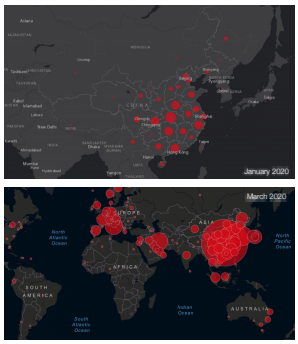
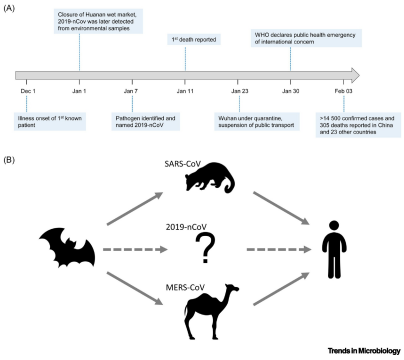
COVID19 epidemics

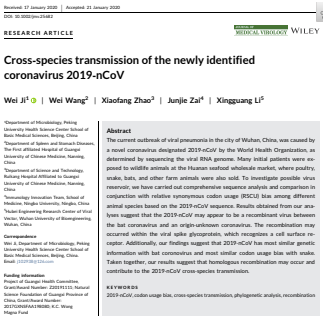
- First cases in December 2019
- Origin in Wuhan city market
- From animal reservoir to inter-human transmission
- January 30 WHO declaration of PHEIC (Public Health Emergency of International Concern)



Origin of SARS-CoV-2



Cross-species transmission relies on genetic mutations



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RESEARCH ARTICLE

JOURNAL OF MEDICAL VIROLOGY WILEY

Cross-species transmission of the newly identified coronavirus 2019-nCoV

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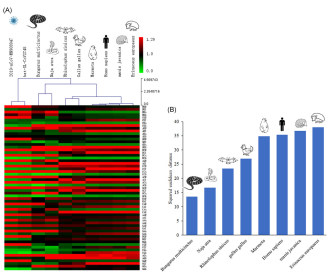
Abstract

The current outbreak of viral pneumonia in the city of Wuhan, China, was caused by a novel coronavirus designated 2019-nCoV by the World Health Organization, as determined by sequencing the viral RNA genome. Many initial patients were exposed to wildlife animals at the Huanan seafood wholesale market, where poultry, snake, bats, and other farm animals were also sold. To investigate possible virus reservoir, we have carried out comprehensive sequence analysis and comparison in conjunction with relative synonymous codon usage (RSCU) bias among different animal species based on the 2019-nCoV sequence. Results obtained from our analyses suggest that the 2019-nCoV may appear to be a recombinant virus between the bat coronavirus and an origin-unknown coronavirus. The recombination may occurred within the viral spike glycoprotein, which recognizes a cell surface receptor. Additionally, our findings suggest that 2019-nCoV has most similar genetic information with bat coronavirus and most similar codon usage bias with snake. Taken together, our results suggest that homologous recombination may occur and contribute to the 2019-nCoV cross-species transmission.

KEYWORDS
2019-nCoV, codon usage bias, cross-species transmission, phylogenetic analysis, recombination



Genetic analysis of SARS-CoV-2

Article

A new coronavirus associated with human respiratory disease in China

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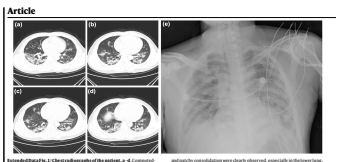
Emerging infectious diseases, such as severe acute respiratory syndrome (SARS) and Zika virus disease, present a major threat to public health[1–3]. Despite intense research efforts, how, when and where new diseases appear are still a source of considerable uncertainty. A severe respiratory disease was recently reported in Wuhan, Hubei province, China. As of 25 January 2020, at least 1,975 cases had been reported since the first patient was hospitalized on 12 December 2019. Epidemiological investigations have suggested that the outbreak was associated with a seafood market in Wuhan. Here we study a single patient who was a worker at the market and who was admitted to the Central Hospital of Wuhan on 26 December 2019 while experiencing a severe respiratory syndrome that included fever, dizziness and a cough. Metagenomic RNA sequencing[4] of a sample of bronchoalveolar lavage fluid from the patient identified a new RNA virus strain from the family Coronaviridae, which is designated here 'Wuhan-Hu-1' coronavirus (and has also been referred to as '2019-nCoV'). Phylogenetic analysis of the complete viral genome (29,903 nucleotides) revealed that the virus was most closely related (89.1% nucleotide similarity) to a group of SARS-like coronaviruses (genus Betacoronavirus, subgenus Sarbecovirus) that had previously been found in bats in China[5]. This outbreak highlights the ongoing ability of viral spill-over from animals to cause severe disease in humans.

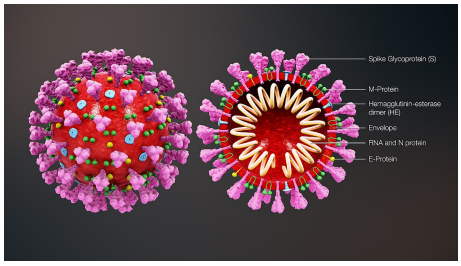


Extended Data Fig. 1 | Chest radiographs of the patient. a–**d**, Computed tomography scans of the chest were obtained on the day of admission (day 1 after the onset of disease). Bilateral focal consolidation, lobar consolidation and patchy consolidation were clearly observed, especially in the lower lung. **e**, A chest radiograph was obtained on day 5 after admission (day 8 after the onset of disease). Bilateral diffuse patchy and fuzzy shadows were observed.

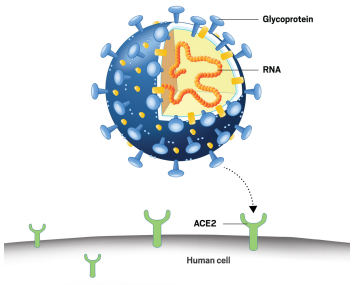
- Similarity between SARS-CoV-1 and SARS-CoV-2

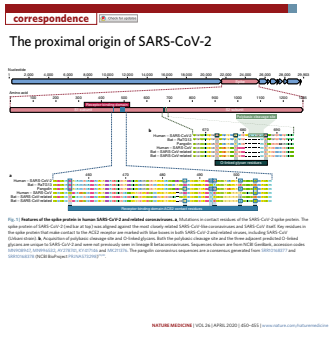
Main characteristics of Coronaviruses

- Replication in the cytoplasm
- Positive-sense, single-stranded RNA genome
- Four structural proteins (**Spike**, Envelope, Membrane, Nucleocapsid)



SARS-CoV-2 binds to human ACE2 receptor

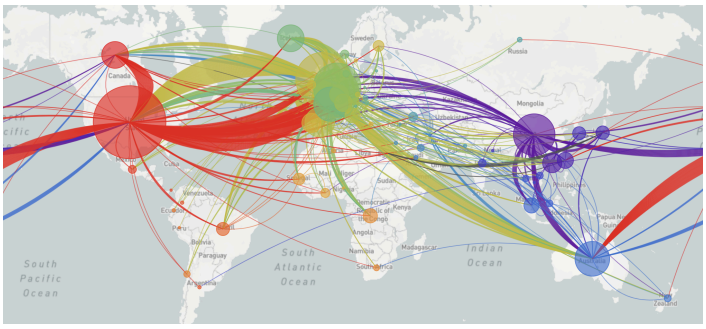




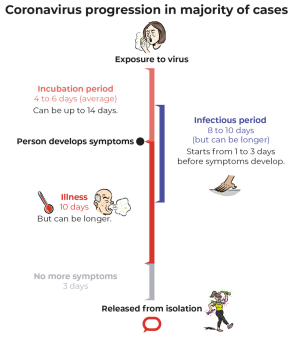
correspondence

The proximal origin of SARS-CoV-2

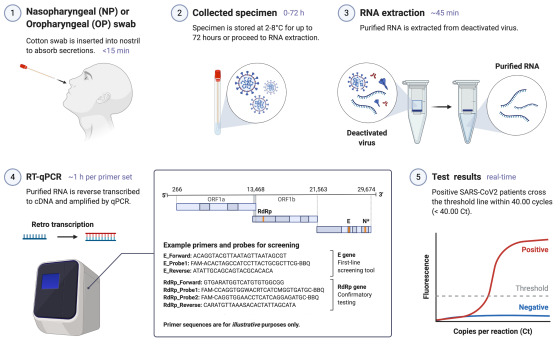
Is SARS-CoV-2 mutating?



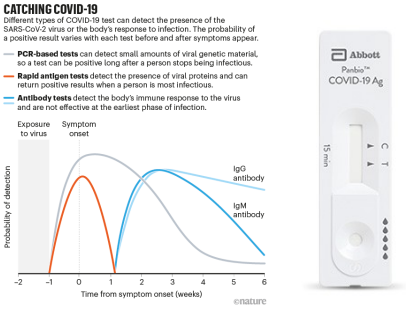
COVID-19 transmission and incubation time



COVID-19 diagnosis

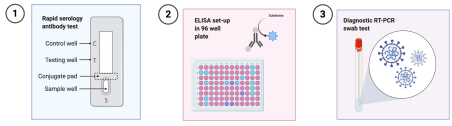


Rapid antigenic tests



Fast coronavirus tests: what they can and can't do *Nature* **585**, 496-498 (2020)

COVID-19 serology?



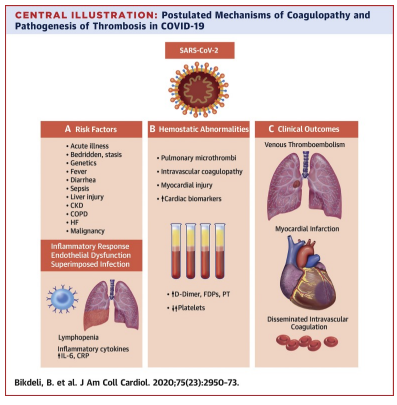
	Rapid serology antibody test	ELISA	Diagnostic RT-PCR swab test
Sample input	**Serum** or **plasma** sample (whole blood or finger prick also possible)	**Serum** or **plasma** sample	**Nasopharyngeal** (NP) or **Oropharyngeal** (OP) **swab** sample
Result output	Detection of **IgM/IgG antibodies** via color change of strip in lateral flow assay	Detection of **IgM/IgG** or RBD IgG **antibodies**, via colorimetric assay	Detection of **viral SARS-CoV-2 RNA** via cDNA sequencing
Strengths	**Very low relative cost**, can be conducted at **point-of-care** or at **home**, ease-of-use, **fast results** (5-15 min, highly accurate detection of IgM/IgG **several days after onset**	**Robust detection of seroconversion** status in a laboratory setting, can detect IgM/IgG highly accurately **several days after onset or sooner**	Gold-standard **diagnostic** test, **directly detects virus** presence (sequencing viral nucleic acids), most **accurate results early** in disease presentation
Limitations	Requires rigorous testing of **cross-reactivity** with other immune response, **variation of test specificity & sensitivity** among manufacturers	Requires rigorous testing of **cross-reactivity** with other immune response, requires **laboratory setting**	**Labor intensive**, requires numerous **additional reagents** and **specialized equipment**, can **lose accuracy** after ~5 days since symptom onset, sensitive to **sample collection error**

COVID-19 pathology

Diffuse alveolare damage

Cytokine storm

Thrombosis



Bikdeli, B. et al. J Am Coll Cardiol. 2020;75(23):2950-73.

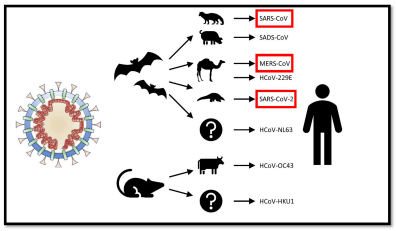
Potential problems to SARS-CoV-2 vaccine development

1. The virus

No effective vaccines have been so far developed for any Coronavirus

Four Coronaviruses are endemic in humans and cause moderate respiratory symptoms.

Two strains caused severe pneumonia, SARS-CoV-1 in 2002-2004 and MERS-CoV in 2012. SARS-CoV-2 belongs to the second group.

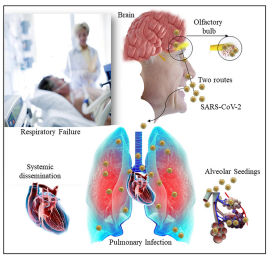


Potential problems to SARS-CoV-2 vaccine development

In most individuals Coronavirus infect upper respiratory tract - the immune system blocks the virus there.

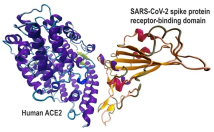
In a few cases, the virus reaches the lung - acquired immunity could protect from complications.

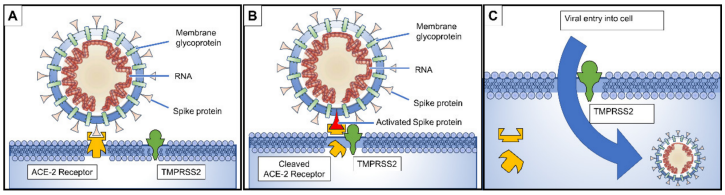
A vaccine could be useless in 80% of cases - it has to have excellent safety profile and would not inhibit transmission.



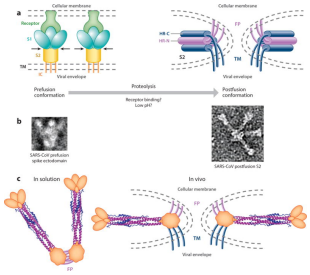
Potential problems to SARS-CoV-2 vaccine development

2. The antigen





Spike



The trimeric S protein contains two subunits, S1 and S2, which mediate receptor binding and fusion, respectively

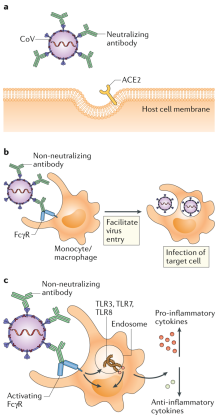
SARS-CoV S protein elicit potent cellular and humoral responses

Other epitopes (i.e. from M, nsp6, ORF3a and N protein) are considered as potential antigens

Potential problems to SARS-CoV-2 vaccine development

3. Induction of neutralising/non neutralising antibodies

ADE: antibody dependent enhancement



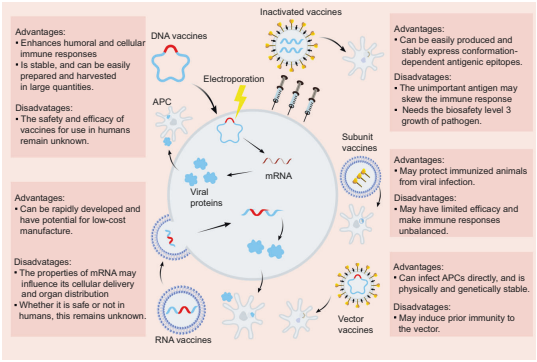
Potential problems to SARS-CoV-2 vaccine development

4. The need of large scale production at an affordable price

Approfondimento Coronavirus

Dopo Londra, anche gli Usa prenotano milioni di dosi del vaccino in sperimentazione tra Oxford e Pomezia. E l'Italia?

Potential SARS-CoV-2 vaccines



Advantages:
- Enhances humoral and cellular immune responses
- Is stable, and can be easily prepared and harvested in large quantities.

Disadvatages:
- The safety and efficacy of vaccines for use in humans remain unknown.

Advantages:
- Can be rapidly developed and have potential for low-cost manufacture.

Disadvatages:
- The properties of mRNA may influence its cellular delivery and organ distribution
- Whether it is safe or not in humans, this remains unknown.

Advantages:
- Can be easily produced and stably express conformation-dependent antigenic epitopes.

Disadvatages:
- The unimportant antigen may skew the immune response
- Needs the biosafety level 3 growth of pathogen.

Advantages:
- May protect immunized animals from viral infection.

Disadvatages:
- May have limited efficacy and make immune responses unbalanced.

Advantages:
- Can infect APCs directly, and is physically and genetically stable.

Disadvatages:
- May induce prior immunity to the vector.

Landscape of COVID-19 candidate vaccines

16 june 2020

11 candidate vaccines in clinical evaluation

Platform	Type of candidate	Developer	Current stage	Same platform for other viruses
Non replicating viral vector	ChAdOx1-S	University of Oxford/ Astrazeneca	Phase 2b/3	MERS, influenza, TB, Chikungunya, Zika, MenB, plague
Non replicating viral vector	Adenovirus Type 5 vector	CanSino Biological Inc/Beijing Institute of Biotechnology	Phase 2	Ebola
RNA	LNP-encapsulated mRNA	MODERNA/NIAID	Phase 2	multiple candidates
Inactivated	Inactivated	Wuhan Institute of Biological Products/Sinopharm	Phase 1/2	
Inactivated	Inactivated	Beijing Institute of Biological Products/Sinopharm	Phase 1/2	
Inactivated	Inactivated + alum	Sinovac	Phase 1/2	SARS
Protein subunit	Recombinant SARS CoV-2 glycoprotein nanoparticle adjuvanted with Matrix M	Novavx	Phase 1/2	RSV; CCHF, HPV, VZV, EBOV
RNA	3 LNP-mRNAs	BioNTech/Fosun Pharma/ Pfizer	Phase 1/2	
Inactivated	Inactivated	Institute of Medical Biology, Chinese Academy of Medical Sciences	Phase 1	
DNA	DNA plasmid with electroporation	Inovio Pharmaceuticals	Phase 1	multiple candidates
RNA	saRNA	Imperial College London	Phase 1	EBOV, LASV, MARV, Inf, RABV

source: WHO

Landscape of COVID-19 candidate vaccines

3 november 2020

47 candidate vaccines in clinical evaluation

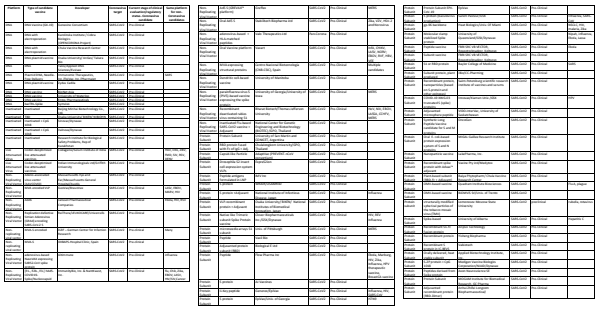
Platform	Type of candidate	Developer	Current stage	Same platform for other viruses
Non replicating viral vector	ChAdOx1-S	University of Oxford/ Astrazeneca	Phase 3	MERS, influenza, TB, Chikungunya, Zika, MenB, plague
Non replicating viral vector	Adenovirus Type 5 vector	CanSino Biological Inc/Beijing Institute of Biotechnology	Phase 3	Ebola
RNA	LNP-encapsulated mRNA	MODERNA/NIAID	Phase 3	multiple candidates
Inactivated	Inactivated	Wuhan Institute of Biological Products/Sinopharm	Phase 3	
Inactivated	Inactivated	Beijing Institute of Biological Products/Sinopharm	Phase 3	
Inactivated	Inactivated + alum	Sinovac	Phase 3	SARS
Protein subunit	Recombinant SARS CoV-2 glycoprotein nanoparticle adjuvanted with Matrix M	Novavx	Phase 3	RSV; CCHF, HPV, VZV, EBOV
RNA	3 LNP-mRNAs	BioNTech/Fosun Pharma/ Pfizer	Phase 3	
Inactivated	Inactivated	Institute of Medical Biology, Chinese Academy of Medical Sciences	Phase 2	
DNA	DNA plasmid with electroporation	Inovio Pharmaceuticals	Phase 2	multiple candidates
RNA	saRNA	Imperial College London	Phase 1	EBOV, LASV, MARV, Inf, RABV
Non replicating viral vector	Adeno-based (rAd26-S+rAd5-S)	Gamaleya Research Institute	Phase 3	Ebola

source: WHO

Landscape of COVID-19 candidate vaccines

16 june 2020

128 candidate vaccines in pre-clinical evaluation

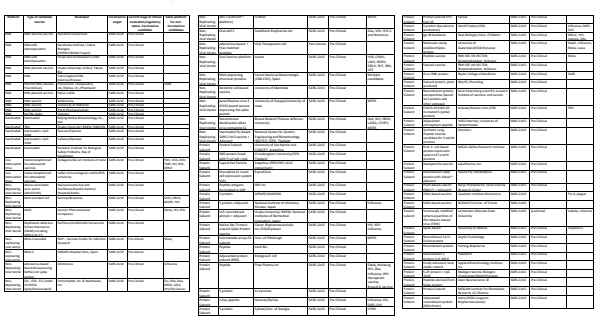


source: WHO

Landscape of COVID-19 candidate vaccines

3 november 2020

155 candidate vaccines in pre-clinical evaluation

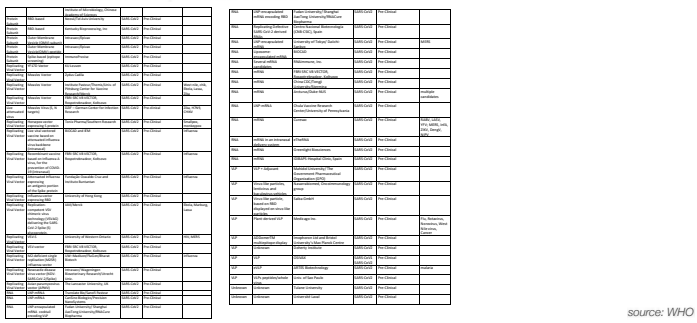


source: WHO

Landscape of COVID-19 candidate vaccines

16 june 2020

128 candidate vaccines in pre-clinical evaluation



source: WHO

Monoclonal antibodies

1. Un topo viene immunizzato mediante iniezione di un antigene X per stimolare la produzione di un anticorpo diretto contro l'antigene X
2. Le cellule che producono anticorpi vengono isolate dalla milza del topo
3. Gli anticorpi monoclonali vengono prodotti fondendo una singola cellula che produce anticorpi con cellule di mieloma (HGPRT negative)
4. La cellula risultante è denominata ibridoma. Ogni ibridoma produce quantità relativamente elevate della medesima molecola anticorpale
5. Facendo moltiplicare l'ibridoma in coltura è possibile ottenere una popolazione di cellule che producono tutte lo stesso anticorpo.

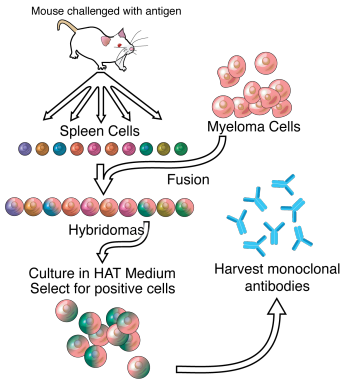


Table 1 | SARS-CoV-2 mAbs in clinical development

Sponsor	Product	Clinical stage	Trial ID	Study
Regeneron/NIAID	REGN-COV2 (REGN10933 + REGN10987; human IgG1 mAbs targeting S protein epitope)	Phase 2/3	NCT04452318	2,000 healthy adults with infected people in household
Regeneron/NIAID	REGN-COV2	Phase 2/3	NCT04426695	2,970 hospitalized adults with COVID-19
Regeneron/NIAID	REGN-COV2	Phase 1/2	NCT04425629	2,104 ambulatory patients with COVID-19
AbCellera/Eli Lilly/NIH	LY3819253 or LY3819253 + LY3832479 (human IgG1 mAbs targeting S protein epitope)	Phase 3	NCT04427501	800 patients with mild to moderate COVID-19
AbCellera/Eli Lilly/NIH	LY3819253 or LY3819253 + LY3832479	Phase 3	NCT04497987	2,400 healthy staff or residents of skilled nursing facilities
AbCellera/Eli Lilly/NIH	LY3819253 versus remdesivir (small-molecule nucleotide analog antiviral that blocks viral RNA polymerase)	Phase 3	NCT04501978	10,000 hospitalized patients
Vir Biotechnology/GlaxoSmithKline	VIR-7831/GSK4182136 (human IgG1 mAb targeting S protein epitope)	Phase 3	NCT04545060	1,360 non-hospitalized patients with COVID-19 at high risk
BeiGene/Singlomics/Peking University	BGB-DXP593 (human IgG1 mAb targeting S protein epitope)	Phase 2	NCT04551898	180 patients with mild to moderate COVID-19
BeiGene/Singlomics/Peking University	BGB-DXP593	Phase 1	NCT04532294	30 healthy adults 18–60 years olds
Junshi Biosciences/Institute of Microbiology, Chinese Academy	JS016 (human mAb targeting S protein epitope)	Phase 1	NCT04441918	40 healthy participants 15–45 years old
Tychan	TY027	Phase 1	NCT04429529	32 healthy adults 21–50 years old
Celltrion	CT-P59 (human mAb targeting S protein epitope)	Phase 1	NCT04525079	32 healthy adults 19–55 years old
Bri Bio/TSB Therapeutics/Tsinghua University	BRI-196 (human mAb targeting S protein epitope)	Phase 1	NCT04479631	12 healthy adults 18–49 years old
Bri Bio/TSB Therapeutics/Tsinghua University	BRI-198 (human mAb targeting S protein epitope)	Phase 1	NCT04479644	12 healthy adults 18–49 years old
Sinocelltech/Chinese Academy of Sciences	SCTA01 (humanized mAb targeting S protein epitopes)	Phase 1	NCT04483375	33 healthy adults
Mabwell (Shanghai) Bioscience	MW33 (human mAb targeting S protein epitope)	Phase 1	NCT04539048	42 healthy adults 18–45 years old
Sorrento/Mount Sinai	COVI-GUARD/STI-1499 (human mAb targeting S1 subunit of S protein)	Phase 1	NCT04454398	33 hospitalized patients with moderate COVID-19
AstraZeneca/Vanderbilt	AZD8895 + AZD1061 (IgG1 human mAbs targeting S protein epitopes)	Phase 1	NCT04507256	48 healthy adults 18–55 years old
Hengenix Biotech	HL-YT0 (human mAb targeting S protein epitope)	Phase 1	NCT04561076	24 healthy adults 18–60 years old

Source: https://www.antibodysociety.org/covid-19-biologics-tracker/

Why are there few antiviral mAb programs compared with other therapeutic areas?

Justification for mAb profilaxis is when there is no effective vaccine (RSV, Ebola) or for populations who do not mount strong immune response (infants and old individuals)

Many viral infections do not represent large markets

In COVID-19 the screening of memory B cells from recovered cases finds many mAbs that bind to S protein and its RBD but neutralising mAb are of lower frequency

What aspects of the antibody-mediated response to coronaviruses have informed mAb engineering?

Antibodies to S protein and RBD induced in patients can neutralise SARS-CoV-2 infectivity in vitro

Knowledge is limited on IgA in mucosal secretions

There is little information on the timeline of antibody response and if antibodies aside from those that bind the S protein play a role in protection

Convalescent plasma may aid early in the course of the disease, based on ongoing observational studies, but controlled trials are needed

Why are S protein and its RBD the sole focus for COVID-19 mAb programs?

Antibodies to S protein and RBD induced in patients can neutralise SARS-CoV-2 infectivity in vitro

Knowledge is limited on IgA in mucosal secretions

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