

Review Article
Global Health



OPEN ACCESS

Received: Feb 7, 2021
Accepted: Apr 15, 2021

Address for Correspondence:
Wenping Gong, PhD

Tuberculosis Prevention and Control Key Laboratory/Beijing Key Laboratory of New Techniques of Tuberculosis Diagnosis and Treatment, Institute for Tuberculosis Research, 8th Medical Center, Chinese PLA General Hospital, #17 Heishanhu Road, Haidian District, Beijing 100091, China.
E-mail: gwp891015@whu.edu.cn

© 2021 The Korean Academy of Medical Sciences.

This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

ORCID iDs

Zaixing Jia
<https://orcid.org/0000-0003-1648-2430>
Wenping Gong
<https://orcid.org/0000-0002-0333-890X>

Funding

This study was funded by the National Natural Science Foundation of China (grant No. 81801643), Beijing Municipal Science and Technology Commission (grant No. 19L2152), and the Chinese PLA General Hospital (grant No. QNC19047).

Will Mutations in the Spike Protein of SARS-CoV-2 Lead to the Failure of COVID-19 Vaccines?

Zaixing Jia and Wenping Gong

Tuberculosis Prevention and Control Key Laboratory/Beijing Key Laboratory of New Techniques of Tuberculosis Diagnosis and Treatment, Institute for Tuberculosis Research, 8th Medical Center, Chinese PLA General Hospital, Beijing, China

ABSTRACT

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the causative agent of coronavirus disease 2019 (COVID-19), which has spread worldwide since it was first identified in Wuhan, China, at the end of 2019. With the global transmission of the virus, a large number of SARS-CoV-2 variants have also appeared, especially, emerging strains that have recently been discovered in the United Kingdom (variant 20I/501Y.V1, lineage B.1.1.7), South Africa (variant 20H/501Y.V2, lineage B.1.351), and Brazil (variant 20J/501Y.V3, and lineage P.1). The common feature of these variants is that they share the N501Y mutation involving the SARS-CoV-2 spike (S) protein, which is precisely the target of most COVID-19 vaccines. Furthermore, mutations such as N501Y, E484K, and K417N in the S protein may affect viral fitness and transmissibility. However, current research on the impact of these variants on COVID-19 vaccines is still lacking. Herein, we briefly explain why most COVID-19 vaccines target the S protein, update the progress of research regarding S protein-related COVID-19 vaccines, review the latest studies concerning the effects of S protein variants on COVID-19 vaccines, and finally, propose certain strategies to deal with SARS-CoV-2 variants.

Keywords: SARS-CoV-2; COVID-19; Spike Protein; Mutation; Variant; Vaccine

Introduction

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Globally, as of April 12, 2021, there have been 135,446,538 confirmed cases of COVID-19, including 2,927,922 deaths, reported to the World Health Organization (WHO, <https://covid19.who.int/>). Vaccination is the best way to fight SARS-CoV-2 infection. To the best of our knowledge, more than eight COVID-19 vaccines have been approved for vaccination among priority groups under an Emergency Use Authorization (EUA), including the Moderna mRNA-1273 vaccine,^{1,2} Pfizer-BioNtech BNT162b2 vaccine,³ China's CoronaVac™ and Sinopharm's COVID-19 vaccines, Russia's Sputnik V and EpiVacCorona vaccines,⁴ AstraZeneca's ChAdOx1 novel coronavirus 2019 (nCoV-19),⁵ and Janssen's Ad26.COV2.S.⁶

Disclosure

The authors declare no conflicts of interest. The funders had no role in the study design; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Author Contributions

Conceptualization: Gong W. Funding acquisition: Gong W. Investigation: Gong W, Jia Z. Methodology: Gong W, Jia Z. Project administration: Gong W. Software: Jia Z. Visualization: Gong W. Writing - original draft: Gong W. Writing - review & editing: Gong W.

Although the EUA of vaccines has brought hope to people under threat of the COVID-19 pandemic, the emergence of SARS-CoV-2 variants at the end of 2020 has rendered the situation confusing. On December 19, 2020, the British government imposed a level 4 blockade on some parts of England due to the spread of the SARS-CoV-2 variant 20I/501Y.V1 (lineage B.1.1.7).⁷ At the end of December, the SARS-CoV-2 variant 20H/501Y.V2 (lineage B.1.351) appeared in South Africa.⁸ On January 2021, the SARS-CoV-2 variant 20J/501Y.V3 (lineage P.1, a branch of the B.1.1.28 lineage) appeared in Brazil, which again attracted attention. The UK SARS-CoV-2 B.1.1.7 variant is defined by multiple spike (S) protein changes (deletion 69-70, deletion 145, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H). The SARS-CoV-2 B.1.351 variant in South Africa has eight mutations involving the S protein (L18F, D80A, D215G, R246I, K417N, E484K, N501Y, and A701V). Unlike the B.1.1.7 lineage, 20H/501Y.V2 (B.1.351 lineage) does not contain the deletion at 69-70. The SARS-CoV-2 P.1 variant in Brazil harbors three S protein mutations E484K, K417N and N501Y in common with 20J/501Y.V2 (lineage B.1.351).

Therefore, people cannot help but ask: why is the S protein essential for vaccine design? Can emergent SARS-CoV-2 variants escape immunity induced by COVID-19 vaccines? How should COVID-19 vaccines cope with SARS-CoV-2 variants?

Why Is the S Protein the Target of Most COVID-19 Vaccine Designs?

The S protein, the most crucial surface protein of SARS-CoV-2, is a large trimeric transmembrane glycoprotein with many glycosylation modifications that form a unique corolla structure on the surface of the virus. The S protein consists of the S1 and S2 subunits (Fig. 1). The S1 subunit mainly contains the receptor-binding domain (RBD), which is responsible for recognizing receptors. Mutations involving RBD-associated amino acids lead to changes in the preference and infection characteristics of viral species. The S2 subunit comprises elements that are essential for membrane fusion. The S protein plays a vital role in the binding of the virus to, and its fusion with, the host cell membrane receptor.¹⁰ Given its crucial role in SARS-CoV-2 infection and adaptive immunity, the S protein is an important target site for neutralizing antibodies and a key target for vaccine design.

As of January 19, 2021, according to the COVID-19 Candidate Vaccine Landscape released by the WHO (<https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines>), there are 64 COVID-19 candidate vaccines in clinical trials (Supplementary Table 1) and 171 COVID-19 candidate vaccines in pre-clinical trials (Supplementary Table 2). Among the 64 COVID-19 vaccines in clinical trials, 44 are based on the S protein, of which 29 are based on the full-length S protein (65.91%, 29/44), 14 on the S protein RBD (31.82%, 14/44), and 1 on the S protein S-2P (2.27%, 1/44) (Table 1). Furthermore, both the Moderna mRNA-1273 vaccine and Pfizer-BioNTech BNT162b2 vaccines were designed based on the nucleotide sequence of the S protein.

What Are the Key Mutations of the S Protein?

Since its discovery in Wuhan in 2019, SARS-CoV-2 has evolved into several co-circulating variants. In order to discuss these variants conveniently, they were divided into 12 major

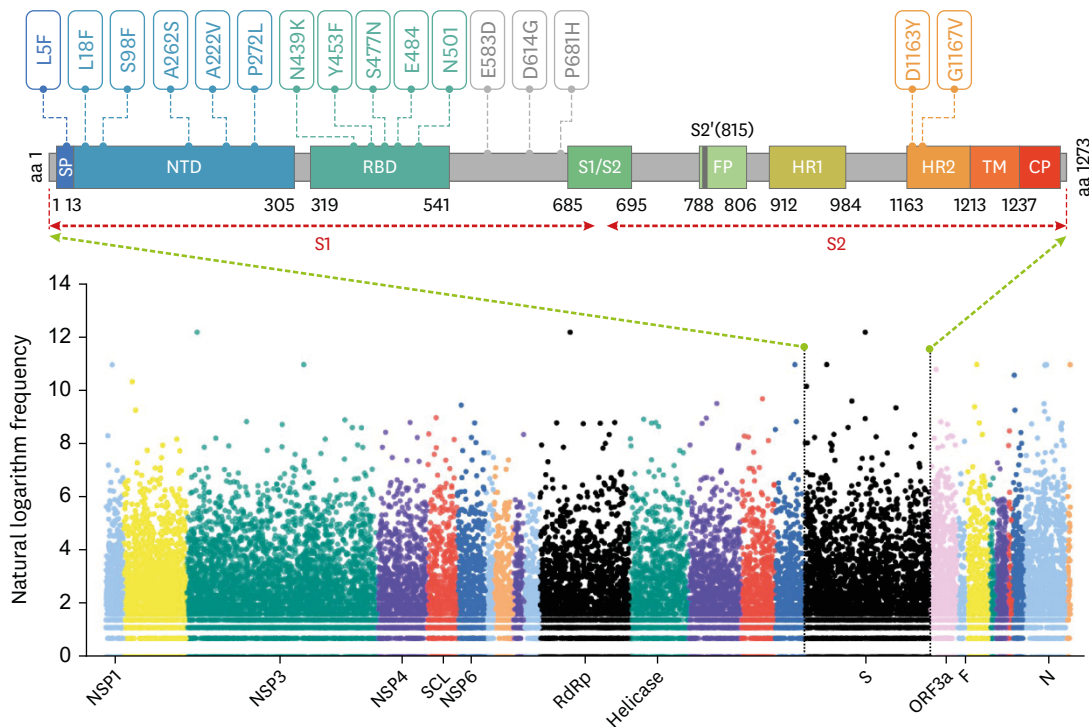


Fig. 1. The distribution of genome-wide mutations on SARS-CoV-2 and the key mutations in S protein. The data of mutations in hCoV-19 genomes was obtained from GISAID database from January 1, 2020, to December 26, 2020. The Y-axis represents the natural logarithm frequency of each mutation in the whole genome of SARS-CoV-2. The X-axis represents the name of the marker gene or protein, and the relative positions of other unlabeled genes or proteins can be found in the GISAID database. Each mutation was shown as a solid dot colored by Open Reading Frame of hCoV-19 genome. The S protein structure was presented following a previous study⁹ (Wrapp et al. *Science* 2020;367:1260-3). The Arabic numerals below the S protein structure represent the amino acid site in the full-length amino acid sequence.

SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2, S = spike, hCoV-19 = human coronavirus 2019.

clades according to specific signature mutations, including 19A and 19B, which emerged in Wuhan, 20A, which arose from 19A and dominated the European outbreak in March 2020, 20B and 20C, which emerged in early 2020, and 20D to 20J, which emerged in the summer of 2020 (Fig. 2A). By analyzing the genomic epidemiology of SARS-CoV-2 obtained from nextstrain.org (<https://nextstrain.org/ncov/global>), we found that the viral mutations were more likely to occur in spring, autumn, and winter, and less likely in summer (as shown by white lines in Fig. 2A). This may be related to the slow spread of the virus in summer due to higher temperatures, as demonstrated by a temporal multivariate time series model in a recent study.¹¹ In addition, we found that the number of mutants in the 20I/501Y.V1, 20H/501Y.V2, and 20J/501Y.V3 clades were higher than those in other clades (Fig. 2B).

To determine mutations in SARS-CoV-2 gene sequences, we obtained, sequenced, and shared SARS-CoV-2 genomic data from the Nextstrain team (<https://nextstrain.org/sars-cov-2/>) and GISAID Mutation Tracker (https://users.math.msu.edu/users/weig/SARS-CoV-2_Mutation_Tracker.html). It was found that as many as 26,844 single mutations were tracked in 203,346 human coronavirus 2019 (hCoV-19) genomes, and the most frequent mutations were observed to involve the NSP3 and S proteins (Fig. 1). Considering the importance of the S protein, we further focused on S protein mutations. By December 26, 2020, at least 5,003 mutations were identified in the S protein by Mutation Tracker (Supplementary Table 3). There were 13 nonsynonymous mutations with a total frequency of > 1,000, and three mutations that are of interest, including D614G, A222V, L18F, S477N, N439K, S98F, L5F,

Spike Protein Mutation is a Challenge for Vaccine Development

Table 1. Landscape of clinical development of COVID-19 candidate vaccines based on S protein

ID	Vaccine platform acronym ^a	Main component	Type of candidate vaccine	No. of doses	Dosing schedule	Route of administration ^b	Developers	Phase
1	DNA	S protein	INO-4800+electroporation	2	Day 0 + 28	ID	Inovio Pharmaceuticals + International Vaccine Institute + Advaccine (Suzhou) Biopharmaceutical Co., Ltd	Phase 2/3
2	DNA	S protein	GX-19	2	Day 0 + 28	IM	Genexine Consortium	Phase 1/2
3	DNA	S protein	CORVax - S Protein Plasmid DNA Vaccine	2	Day 0 + 14	ID	Providence Health & Services	Phase 1
4	DNA	S protein	bacTRL-S oral DNA vaccine	1	Day 0	Oral	Symvivo Corporation	Phase 1
5	PS	RBD of S protein	Recombinant SARS-CoV-2 vaccine (CHO cell)	2-3	Day 0 + 28 or Day 0 + 28 + 56	IM	Anhui Zhifei Longcom Biopharmaceutical + Institute of Microbiology, Chinese Academy of Sciences	Phase 3
6	PS	S protein	SARS-CoV-2 rS/Matrix M1-Adjuvant (full length recombinant SARS CoV-2 glycoprotein nanoparticle vaccine adjuvanted with Matrix M)	2	Day 0 + 21	IM	Novavax	Phase 3
7	PS	RBD of S protein	UB-612 (Multitope peptide based S1-RBD-protein based vaccine)	2	Day 0 + 28	IM	COVAXX + United Biomedical Inc	Phase 2/3
8	PS	S-2P protein	MVC-COV1901 (S-2P protein + CpG 1018)	2	Day 0 + 28	IM	Medigen Vaccine Biologics + Dynavax + National Institute of Allergy and Infectious Diseases (NIAID)	Phase 2/3
9	PS	S protein	SCB-2019 + AS03 or CpG 1018 adjuvant plus Alum adjuvant (native like trimeric subunit S protein vaccine)	2	Day 0 + 21	IM	Clover Biopharmaceuticals Inc./ GSK/Dynavax	Phase 2/3
10	PS	RBD of S protein	FINLAY-FR anti-SARS-CoV-2 Vaccine (RBD + adjuvant)	2	Day 0 + 28	IM	Instituto Finlay de Vacunas	Phase 2
11	PS	RBD of S protein	RBD (baculovirus production expressed in Sf9 cells) Recombinant SARS-CoV-2 vaccine (Sf9 cell)	2	Day 0 + 28	IM	West China Hospital + Sichuan University	Phase 2
12	PS	RBD of S protein	KBP-COVID-19 (RBD-based)	2	Day 0 + 21	IM	Kentucky Bioprocessing Inc.	Phase 1/2
13	PS	RBD of S protein	CIGB-669 (RBD + AgnHB)	3	Day 0 + 14 + 28 or Day 0 + 28 + 56	IN	Center for Genetic Engineering and Biotechnology (CIGB)	Phase 1/2
14	PS	RBD of S protein	CIGB-66 (RBD + aluminium hydroxide)	3	Day 0 + 14 + 28 or Day 0 + 28 + 56	IM	Center for Genetic Engineering and Biotechnology (CIGB)	Phase 1/2
15	PS	RBD of S protein	BECOV2	2	Day 0 + 28	IM	Biological ELimited	Phase 1/2
16	PS	RBD of S protein	SARS-CoV-2-RBD-Fc fusion protein			SC or IM	University Medical Center Groningen + Akston Biosciences Inc.	Phase 1/2
17	PS	S protein	SARS-CoV-2 vaccine formulation 1 with adjuvant 1 (S protein baculovirus production)	2	Day 0 + 21	IM	Sanofi Pasteur + GSK	Phase 1/2
18	PS	S protein	Recombinant SARS-CoV-2 S protein, aluminum adjuvanted	2	Day 0 + 21	IM	Nanogen Pharmaceutical Biotechnology	Phase 1/2
19	PS	S protein	COVAC-1 and COVAC-2 subunit vaccine (S protein) + SWE adjuvant	2	Day 0 + 28	IM	Vaccine and Infectious Disease Organization (VIDO) + Seppic and the Vaccine Formulation Institute (VFI)	Phase 1/2
20	PS	RBD of S protein	AdimrSC-2f (recombinant RBD ± aluminium)	ND	ND	ND	Adimmune Corporation	Phase 1
21	PS	S protein	COVAX-19 [®] recombinant S protein + adjuvant	1	Day 0	IM	Vaxine Pty Ltd.	Phase 1
22	RNA	S protein	mRNA-1273	2	Day 0 + 28	IM	Moderna + National Institute of Allergy and Infectious Diseases (NIAID)	Phase 3
23	RNA	S protein	CV nCoV vaccine	2	Day 0 + 28	IM	CureVac AG	Phase 3
24	RNA	S protein	BNT162b2 (3 LNP-mRNAs)	2	Day 0 + 21	IM	Pfizer/BioNTech + Fosun Pharma	Phase 2/3

(continued to the next page)

Table 1. (Continued) Landscape of clinical development of COVID-19 candidate vaccines based on S protein

ID	Vaccine platform acronym ^a	Main component	Type of candidate vaccine	No. of doses	Dosing schedule	Route of administration ^b	Developers	Phase
25	RNA	S protein	ARCT-021	ND	ND	IM	Arcturus Therapeutics	Phase 2
26	RNA	RBD of S protein	SARS-CoV-2 mRNA vaccine (ARCoV)	2	Day 0 + 14 or Day 0 + 28	IM	Academy of Military Science (AMS), Walvax Biotechnology and Suzhou Abogen Biosciences	Phase 1
27	RNA	S protein	LNP-nCoVsaRNA	2	ND	IM	Imperial College London	Phase 1
28	RNA	S protein	ChulaCov19 mRNA vaccine	2	Day 0 + 21	IM	Chulalongkorn University	Phase 1
29	VLP	S protein	Coronavirus-Like Particle COVID-19 (CoVLP)	2	Day 0 + 21	IM	Medicago Inc.	Phase 2/3
30	VLP	RBD of S protein	RBD SARS-CoV-2 HBsAg VLP vaccine	2	Day 0 + 28	IM	Serum Institute of India + Accelagen Pty + SpyBiotech	Phase 1/2
31	VVnr	S protein	ChAdOx1 nCoV-19/AZD1222 vaccine (Covishield)	1-2	Day 0 + 28	IM	AstraZeneca + University of Oxford	Phase 3
32	VVnr	S protein	Recombinant novel coronavirus vaccine (adenovirus type 5 vector)	1	Day 0	IM	CanSino Biological Inc./Beijing Institute of Biotechnology	Phase 3
33	VVnr	S protein	Gam-COVID-Vac Adeno-based (rAd26-S+rAd5-S)	2	Day 0 + 21	IM	Gamaleya Research Institute; Health Ministry of the Russian Federation	Phase 3
34	VVnr	S protein	Ad26.COV2.S	1-2	Day 0 or Day 0 + 56	IM	Janssen/Johnson & Johnson	Phase 3
35	VVnr	RBD of S protein	AdCOVID, adenovirus-based platform expresses the RBD of the SARS-CoV-2 S protein	1-2	Day 0	IN	Altimmune, Inc.	Phase 1
36	VVnr	S-Fusion+N-ETSD	hAd5-S-Fusion+N-ETSD vaccine	1	Day 0	Oral	ImmunityBio, Inc.	Phase 1
37	VVnr	S protein	GRAd-COV2 (replication defective simian adenovirus [GRAd] encoding S)	1	Day 0	IM	ReiThera + Leukocare + Univercells	Phase 1
38	VVnr	S protein	MVA-SARS-2-S	2	Day 0 + 28	IM	University of Munich (Ludwig-Maximilians)	Phase 1
39	VVnr	S protein	COH04S1 (MVA-SARS-2-S) - Modified vaccinia ankara (sMVA) platform + synthetic SARS-CoV-2	1-2	Day 0 + 28	IM	City of Hope Medical Center + National Cancer Institute	Phase 1
40	VVr	RBD of S protein	DelNS1-2019-nCoV-RBD-OPT1 (intranasal flu-based-RBD)	1	Day 0	IN	University of Hong Kong, Xiamen University and Beijing Wantai Biological Pharmacy	Phase 2
41	VVr	S protein	V591-001-Measles-vector based (TMV-038)	1-2	Day 0 + 28	IM	Merck & Co. + Themis + Sharp & Dohme + Institute Pasteur + University of Pittsburgh	Phase 1/2
42	VVr	S protein	rVSV-SARS-CoV-2-S vaccine	1	Day 0	IM	Israel Institute for Biological Research	Phase 1/2
43	VVr	S protein	AdCLD-CoV19 (adenovirus vector)	1	Day 0	IM	Cellid Co., Ltd.	Phase 1/2
44	VVr + APC	S protein	Dendritic cell vaccine AV-COVID-19. A vaccine consisting of autologous dendritic cells loaded with antigens from SARS-CoV-2, with or without GM-CSF	1	Day 0	IM	Aivita Biomedical, Inc. National Institute of Health Research and Development, Ministry of Health Republic of Indonesia	Phase 1/2

COVID-19 = coronavirus disease 2019, S = spike, RBD = receptor-binding domain, SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2, GM-CSF = granulocyte-macrophage colony-stimulating factor, ND = no data.

^aVaccine platform acronyms are follow as: DNA = DNA based vaccine, PS = protein subunit, RNA = RNA based vaccine, VLP = virus like particle, VVnr = viral vector (non-replicating), VVr = viral vector (replicating), APC = antigen-presenting cell. ^bAbbreviations of route of administration are follow as: ID = intradermal injection, IM = intramuscular injection, IN = intranasal spray, Oral = orally delivered, SC = subcutaneous injection.

A262S, P272L, P681H, D1163Y, E583D, G1167V, Y453F, E484K, and N501Y (**Fig. 1**). Among these mutations, five (L18F, S98F, A262S, A222V, and P272L) occurred in the N-terminal domain (NTD) of the S protein, and five (N439K, Y453F, S477N, E484K, and N501Y) appeared in the S protein RBD.

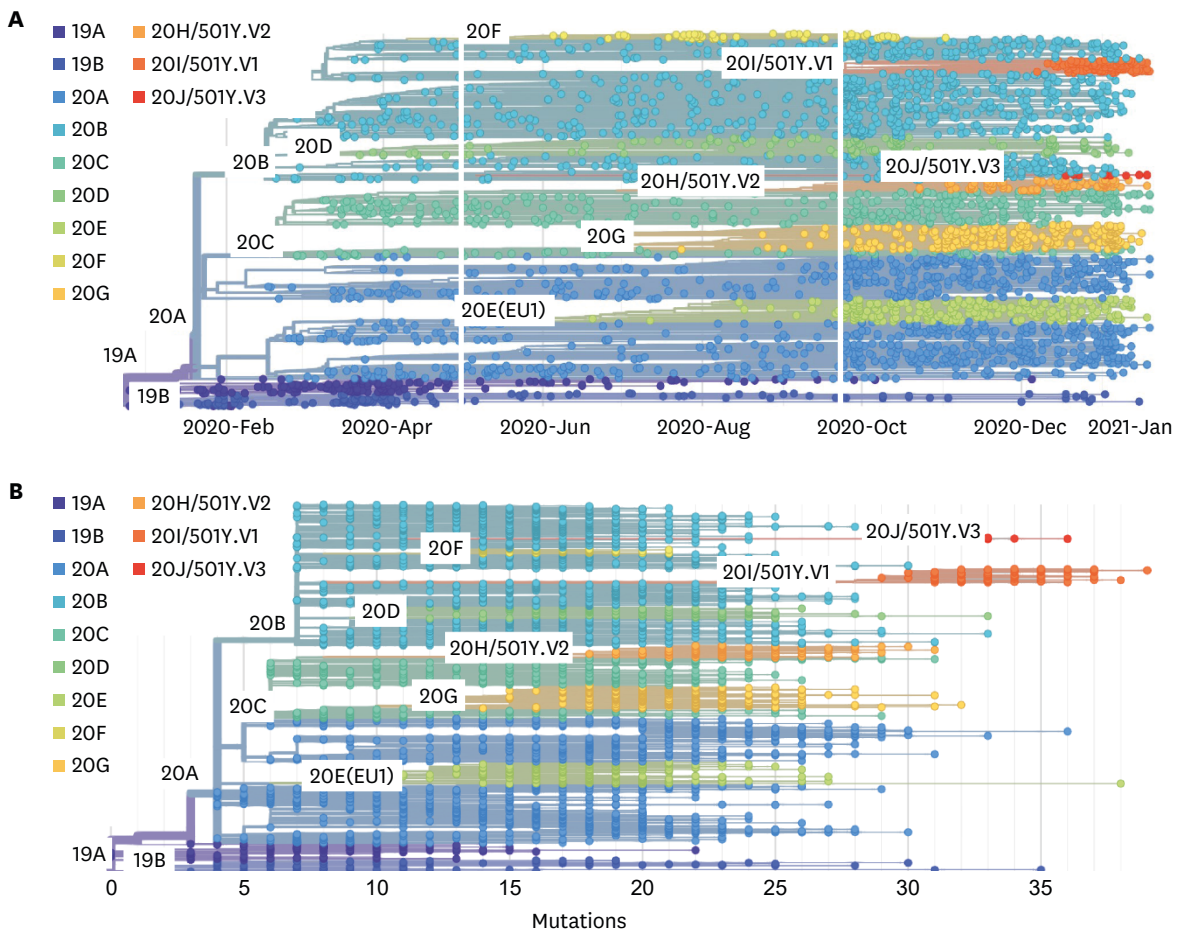


Fig. 2. The phylogeny and mutations of SARS-CoV-2 viruses. This phylogeny shows evolutionary relationships of SARS-CoV-2 viruses from the ongoing COVID-19 pandemic (**A**). Although the genetic relationships among sampled viruses are quite clear, there is considerable uncertainty surrounding estimates of specific transmission dates and in the reconstruction of geographic spread. The number of mutations in hCoV-19 genomes was obtained from Nextstrain database and shown by solid dot (**B**). Compiled Nextstrain SARS-CoV-2 resources are available at <https://nextstrain.org/sars-cov-2/>. All of data was obtained on January 26, 2021. SARS-CoV-2 = severe acute respiratory syndrome coronavirus-2, COVID-19 = coronavirus disease 2019, hCoV-19 = human coronavirus 2019.

Do These Mutations Affect COVID-19 Vaccines Targeting the S Protein?

A large number of previous studies and practices have demonstrated that viral mutations exist naturally, and these mutations rarely affect viral fitness or the effects of vaccines. However, the emergence of mutations in the spike protein of SARS-CoV-2 might impact viral fitness and transmissibility, particularly after the recent identification of three independent emerging strains in the UK (UK, 20I/501Y.V1), South Africa (20H/501Y.V2), and Brazil (20J/501Y.V3).¹² The emergence of these variant strains is remarkable because the N501Y mutation shared by the three strains is located in the key RBD of the S protein.¹³ This mutation may enhance the ability of SARS-CoV-2 to bind to human receptor angiotensin-converting enzyme 2 (ACE2), thus accelerating the spread of the COVID-19 pandemic.^{7,14} A preprint study published in medRxiv showed that serum of convalescent COVID-19 patients and vaccinators could neutralize the N501Y variant, suggesting that current SARS-CoV-2 vaccines will protect against the 20B/501Y.V1 strain.¹⁵

In addition, the D614G, S477N, and E484K mutants of the S protein are considered to pose a risk of immune escape or increased ACE2 binding by the virus, thereby affecting COVID-19 vaccine development and antibody treatment.¹⁶ A previous study reported that the D614G mutation increases susceptibility of the virus to neutralization, and the D614G mutation is not expected to threaten current vaccine development.¹⁷ However, in another study, the D614G mutation did not alter the binding of the spike protein to ACE2 or the neutralization sensitivity of pseudoviruses. The authors suggested that the D614G mutation might increase infectivity by assembling more functional S protein into virions.¹⁸ S477N, a mutation involving the RBD, has emerged independently in Australia and is responsible for much of the summer 2020 outbreak. Previous studies have indicated that this mutation may slightly increase the ACE2 binding affinity¹⁹ of the virus and confer resistance to multiple antibodies.²⁰ The E484K mutation is associated with the 501Y.V2 variant in South Africa and the 501Y.V3 variant identified in Manaus, Amazonas, and Brazil.^{8,12} Greaney et al.²¹ found that E484K reduced the neutralizing potency of convalescent sera from some donors by 10-fold. In one study, following the co-incubation of SARS-CoV-2 with convalescent plasma, the virus was reported to completely escape neutralization by day 73 due to the emergence of the E484 mutation.²²

On January 25, 2021, a preprint study in bioRxiv assessed the neutralizing capacity of sera from human subjects or non-human primates that received the mRNA-1273 vaccine and demonstrated that compared with vesicular stomatitis virus (VSV) pseudovirus, VSV pseudoviruses with S protein containing K417N-E484K-N501Y-D614G resulted in a 2.7-fold higher geometric mean titer reduction.²³ Another study evaluated antibody and memory B cell responses in a cohort of 20 volunteers vaccinated with the Moderna (mRNA-1273) or Pfizer-BioNTech (BNT162b2) vaccines. The results showed that the neutralization activity induced by both vaccines decreased slightly, but nevertheless, significantly, against SARS-CoV-2 variants encoding E484K or N501Y, or the K417N-E484K-N501Y combination.²⁴ On January 26, 2021, a preprint study in bioRxiv reported that Covaxin, the COVID-19 vaccine developed by Bharat Biotech, effectively neutralized the UK variant of SARS-CoV-2, reducing the possibility of immune escape by the mutant virus.²⁵ Similarly, the neutralizing activity of antibodies induced by the ChAdOx1 nCoV-19 vaccine was 9-fold lower against the B.1.1.7 variant than against the canonical non-B. 1.1.7 lineage, but its efficacy against the B.1.1.7 variant (74.6%) was similar to that of the vaccine against other lineages (84%).²⁶ Recently, a preclinical study demonstrated that the Ad26.COVS.S vaccine increased the levels of neutralizing antibodies and provided protection against the SARS-CoV-2 G614 spike variant by performing immune challenge experiments in a Syrian hamster model.²⁷

Interestingly, on January 28, 2021, Novavax, Inc., a biotechnology company developing next-generation vaccines, announced that its COVID-19 vaccine NVX-CoV2373 had completed a Phase III clinical trial in the UK with a protection efficiency of 95.6% against the original COVID-19 strain and 85.6% against the UK variant strain.²⁸ Furthermore, a phase IIb clinical trial of the NVX-CoV2373 vaccine in South Africa showed that the vaccine is 60% effective in preventing the replication of the original strain of COVID-19. However, its effectiveness in preventing the South African variant strain was only 49.4%.²⁸ This is the first vaccine to demonstrate clinical efficacy against 'normal' COVID-19 and both the UK and South African variants.

This evidence indicates that mutations in the S protein have the potential to affect the binding of antibodies to host receptors, leading to a change in the infectivity and transmission efficiency of the virus, and its immune escape from neutralization after vaccination. There is

still a lack of studies involving larger sample sizes to assess the impact of these mutations on COVID-19 vaccines. These issues need to be further investigated experimentally and clinically in the future.

What Measures Should Be Taken to Deal with Viral Mutations?

Taken together, although the studies mentioned above have involved preliminary explorations regarding the protective effects of the approved vaccines against SARS-CoV-2 variants from different perspectives, there is still a lack of in-depth studies and studies with extensive sample sizes. Because we know little about these newly emerging mutants, we are still passive and slow with regard to dealing with these potential emergencies. In the future, the following measures should be taken to meet the challenges associated with SARS-CoV-2 variants:

- (1) The monoclonal antibodies employed in the clinic should be tested again against the new variants. As a COVID-19 therapeutic agent, S protein-specific neutralizing antibodies can interact with the SARS-CoV-2 S protein to inhibit the virus from invading the human body. Monoclonal antibodies have been authorized for emergency use. It has been reported that viral mutants may reduce the effectiveness of neutralizing antibodies.²⁹ Therefore, it is urgent to confirm the efficacy of these monoclonal and serum antibodies in neutralizing mutant viruses.
- (2) Vaccines under EUA may need to be updated periodically with respect to clinical efficacy against SARS-CoV-2 variants. Currently, 44 vaccines that have entered the clinical trial stage were developed based on the S protein, and the data indicate that the S protein is the most mutated part of the SARS-CoV-2 virus. Increasing evidence also shows that some COVID-19 vaccines are less effective in protecting against variants.^{24,28} These data suggest that vaccine manufacturers must update their vaccines in time to deal with viral mutations. Otherwise, the efficacy of the COVID-19 vaccine may be affected.
- (3) The effectiveness of vaccines under EUA should be evaluated against SARS-CoV-2 variants. Phase III clinical trials of the six vaccines currently approved for emergency use were completed before the end of 2020. The actual efficacy data were also obtained based on infection by the original SARS-CoV-2 strain. This means that the efficacy of these vaccines against SARS-CoV-2 variants remains unknown. Thus far, only the efficacy of the NVX-CoV2373 vaccine against variants has been studied in a phase III clinical trial, and the results indicate that the efficacies of the NVX-CoV2373 vaccine against the original COVID-19, the UK variant, and the South African variant strains were 95.6%, 85.6%, and 49.4%, respectively.²⁸
- (4) For vaccines still in pre-clinical studies, SARS-CoV-2 variant-infected animal models should be established to assess their efficacy. Transgenic mice with humanized lungs and immune systems represent ideal animal models for COVID-19 vaccine and drug development.³⁰ In August 2020, Hansen et al.³¹ created a humanized mouse model and compared the similarities and consistencies of antibodies against the SARS-CoV-2 S protein produced by humanized mice and convalescent patients. In the future, the development of a transgenic or humanized animal model for SARS-CoV-2 variant infection will greatly promote the development of more effective COVID-19 vaccines.
- (5) Implement more stringent public health control strategies, such as wearing masks and social distancing. The scientific formulation and rapid and effective implementation of public health control strategies by governments are decisive factors for decreasing viral transmission, especially in countries where vaccines have not yet been made available. If

people strictly abide by these public health control strategies, SARS-CoV-2 transmission would decrease, thereby lowering the frequency of mutations. In the face of the rapidly mutating virus, formulating and following public health control strategies is one of the most effective and economical strategies to deal with such crises.

- (6) Efforts should be made to curb the spread of variant SARS-CoV-2 strains through international cooperation and by strengthening immigration quarantine. Experience and studies indicate that the rapid spread of COVID-19 is closely related to intensive personnel exchanges and cross-border travel.³² What is even more worrying is that variant SARS-CoV-2 viruses tend to have stronger transmission capabilities, which makes the spread of such variants more difficult to control internationally. To circumvent this challenge, effective international cooperation strategies should be formulated and implemented, and at the same time, countries should strengthen their immigration quarantine policies to reduce the risk of the cross-border transmission of SARS-CoV-2 variants.

Conclusions

In summary, mutations of SARS-CoV-2 have presented new challenges to the prevention and treatment of COVID-19. Most COVID-19 vaccines that have been approved for emergency use or are still in clinical research are designed based on the S protein. Unfortunately, the mutation frequency of the S protein is very high, and some mutations (such as E484K, N501Y, and K417N) affect the transmission and neutralization of the SARS-CoV-2 virus. Recently, there have been studies concerning SARS-CoV-2 mutants and EUA vaccines published online on preprint platforms; however, the results of these studies are inconsistent or even contradictory. Thus, surveillance activities, including gene sequencing and evaluation of vaccine effectiveness against SARS-CoV-2 variants, should be strengthened.

ACKNOWLEDGMENTS

We would like to thank Editage (www.editage.cn) for English language editing.

SUPPLEMENTARY MATERIALS

Supplementary Table 1

Landscape of COVID-19 candidate vaccines in clinical development

[Click here to view](#)

Supplementary Table 2

Landscape of COVID-19 candidate vaccines in pre-clinical development

[Click here to view](#)

Supplementary Table 3

Spike frequency obtained from the GISAID database

[Click here to view](#)

REFERENCES

1. Oliver SE, Gargano JW, Marin M, Wallace M, Curran KG, Chamberland M, et al. The Advisory Committee on Immunization Practices' interim recommendation for use of Moderna COVID-19 vaccine - United States, December 2020. *MMWR Morb Mortal Wkly Rep* 2021;69(5152):1653-6.
[PUBMED](#) | [CROSSREF](#)
2. Mahase E. Covid-19: UK approves Moderna vaccine to be given as two doses 28 days apart. *BMJ* 2021;372:n74.
[PUBMED](#) | [CROSSREF](#)
3. Mahase E. Covid-19: UK approves Pfizer and BioNTech vaccine with rollout due to start next week. *BMJ* 2020;371:m4714.
[PUBMED](#) | [CROSSREF](#)
4. Mahase E. Covid-19: Russia approves vaccine without large scale testing or published results. *BMJ* 2020;370:m3205.
[PUBMED](#) | [CROSSREF](#)
5. Voysey M, Costa Clemens SA, Madhi SA, Weckx LY, Folegatti PM, Aley PK, et al. Single-dose administration and the influence of the timing of the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine: a pooled analysis of four randomised trials. *Lancet* 2021;397(10277):881-91.
[PUBMED](#) | [CROSSREF](#)
6. Stephenson KE, Le Gars M, Sadoff J, de Groot AM, Heerwegh D, Truysers C, et al. Immunogenicity of the Ad26.COV2.S vaccine for COVID-19. *JAMA*. Forthcoming 2021. DOI: 10.1001/jama.2021.3645.
[PUBMED](#) | [CROSSREF](#)
7. Galloway SE, Paul P, MacCannell DR, Johansson MA, Brooks JT, MacNeil A, et al. Emergence of SARS-CoV-2 B.1.1.7 lineage - United States, December 29, 2020–January 12, 2021. *MMWR Morb Mortal Wkly Rep* 2021;70(3):95-9.
[PUBMED](#) | [CROSSREF](#)
8. Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J, et al. Emergence and rapid spread of a new severe acute respiratory syndrome-related coronavirus 2 (SARS-CoV-2) lineage with multiple spike mutations in South Africa. *medRxiv*. Forthcoming 2020. DOI: 10.1101/2020.12.21.20248640.
[CROSSREF](#)
9. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science*. 2020;367(6483):1260-3.
[PUBMED](#) | [CROSSREF](#)
10. Duan L, Zheng Q, Zhang H, Niu Y, Lou Y, Wang H. The SARS-CoV-2 spike glycoprotein biosynthesis, structure, function, and antigenicity: implications for the design of spike-based vaccine immunogens. *Front Immunol* 2020;11:576622.
[PUBMED](#) | [CROSSREF](#)
11. Rui R, Tian M, Tang ML, Ho GT, Wu CH. Analysis of the spread of COVID-19 in the USA with a spatio-temporal multivariate time series model. *Int J Environ Res Public Health* 2021;18(2):774.
[PUBMED](#) | [CROSSREF](#)
12. Naveca F, Nascimento V, Souza V, Corado A, Nascimento F, Silva G, et al. Phylogenetic relationship of SARS-CoV-2 sequences from Amazonas with emerging Brazilian variants harboring mutations E484K and N501Y in the spike protein. <https://virological.org/t/phylogenetic-relationship-of-sars-cov-2-sequences-from-amazonas-with-emerging-brazilian-variants-harboring-mutations-e484k-and-n501y-in-the-spike-protein/585>. Updated 2020. Accessed March 19, 2021.
13. GISAID. UK reports new variant, termed VUI 202012/01. <https://www.gisaid.org/references/gisaid-in-the-news/uk-reports-new-variant-termed-vui-20201201/>. Updated 2020. Accessed March 19, 2021.
14. Teruel N, Mailhot O, Najmanovich RJ. Modelling conformational state dynamics and its role on infection for SARS-CoV-2 Spike protein variants. *bioRxiv*. Forthcoming 2020. DOI: 10.1101/2020.12.16.423118.
[CROSSREF](#)
15. Rathnasinghe R, Jangra S, Cupic A, Martínez-Romero C, Mulder LCF, Kehrler T, et al. The N501Y mutation in SARS-CoV-2 spike leads to morbidity in obese and aged mice and is neutralized by convalescent and post-vaccination human sera. *medRxiv*. Forthcoming 2021. DOI: 10.1101/2021.01.19.21249592.
[PUBMED](#) | [CROSSREF](#)
16. Koyama T, Weeraratne D, Snowdon JL, Parida L. Emergence of drift variants that may affect COVID-19 vaccine development and antibody treatment. *Pathogens* 2020;9(5):324.
[PUBMED](#) | [CROSSREF](#)

17. Weissman D, Alameh MG, de Silva T, Collini P, Hornsby H, Brown R, et al. D614G spike mutation increases SARS-CoV-2 susceptibility to neutralization. *Cell Host Microbe* 2021;29(1):23-31.e4.
[PUBMED](#) | [CROSSREF](#)
18. Zhang L, Jackson CB, Mou H, Ojha A, Peng H, Quinlan BD, et al. SARS-CoV-2 spike-protein D614G mutation increases virion spike density and infectivity. *Nat Commun* 2020;11(1):6013.
[PUBMED](#) | [CROSSREF](#)
19. Chen J, Wang R, Wang M, Wei GW. Mutations strengthened SARS-CoV-2 infectivity. *J Mol Biol* 2020;432(19):5212-26.
[PUBMED](#) | [CROSSREF](#)
20. Gaebler C, Wang Z, Lorenzi JCC, Muecksch F, Finkin S, Tokuyama M, et al. Evolution of antibody immunity to SARS-CoV-2. *Nature* 2021;591(7851):639-44.
[PUBMED](#) | [CROSSREF](#)
21. Greaney AJ, Loes AN, Crawford KHD, Starr TN, Malone KD, Chu HY, et al. Comprehensive mapping of mutations to the SARS-CoV-2 receptor-binding domain that affect recognition by polyclonal human serum antibodies. *Cell Host Microbe* 2021;29(3):463-476.e6.
[PUBMED](#) | [CROSSREF](#)
22. Andreano E, Piccini G, Licastro D, Casalino L, Johnson NV, Paciello I, et al. SARS-CoV-2 escape in vitro from a highly neutralizing COVID-19 convalescent plasma. *bioRxiv*. Forthcoming 2020. DOI: 10.1101/2020.12.28.424451.
[PUBMED](#) | [CROSSREF](#)
23. Wu K, Werner AP, Moliva JL, Koch M, Choi A, Stewart-Jones GBE, et al. mRNA-1273 vaccine induces neutralizing antibodies against spike mutants from global SARS-CoV-2 variants. *bioRxiv*. Forthcoming 2021. DOI: 10.1101/2021.01.25.427948.
[PUBMED](#) | [CROSSREF](#)
24. Wang Z, Schmidt F, Weisblum Y, Muecksch F, Barnes CO, Finkin S, et al. mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants. *Nature*. Forthcoming 2021. DOI: 10.1038/s41586-021-03324-6.
[PUBMED](#) | [CROSSREF](#)
25. Sapkal GN, Yadav PD, Ella R, Deshpande GR, Sahay RR, Gupta N, et al. Neutralization of UK-variant VUI-202012/01 with COVAXIN vaccinated human serum. *bioRxiv*. Forthcoming 2021. DOI: 10.1101/2021.01.26.426986.
[CROSSREF](#)
26. Emary KRW, Golubchik T, Aley PK, Ariani CV, Angus B, Bibi S, et al. Efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 variant of concern 202012/01 (B.1.1.7): an exploratory analysis of a randomised controlled trial. *Lancet* 2021;397(10282):1351-62.
[PUBMED](#) | [CROSSREF](#)
27. van der Lubbe JEM, Rosendahl Huber SK, Vijayan A, Dekking L, van Huizen E, Vreugdenhil J, et al. Ad26.COV2.S protects Syrian hamsters against G614 spike variant SARS-CoV-2 and does not enhance respiratory disease. *NPJ Vaccines* 2021;6(1):39.
[PUBMED](#) | [CROSSREF](#)
28. Novavax. Novavax COVID-19 vaccine demonstrates 89.3% efficacy in UK phase 3 trial. <https://ir.novavax.com/news-releases/news-release-details/novavax-covid-19-vaccine-demonstrates-893-efficacy-uk-phase-3>. Updated 2021. Accessed January 28, 2021.
29. Liu Z, VanBlargan LA, Bloyet LM, Rothlauf PW, Chen RE, Stumpf S, et al. Identification of SARS-CoV-2 spike mutations that attenuate monoclonal and serum antibody neutralization. *Cell Host Microbe* 2021;29(3):477-488.e4.
[PUBMED](#) | [CROSSREF](#)
30. Pujhari S, Rasgon JL. Mice with humanized-lungs and immune system - an idealized model for COVID-19 and other respiratory illness. *Virulence* 2020;11(1):486-8.
[PUBMED](#) | [CROSSREF](#)
31. Hansen J, Baum A, Pascal KE, Russo V, Giordano S, Wloga E, et al. Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. *Science* 2020;369(6506):1010-4.
[PUBMED](#) | [CROSSREF](#)
32. Environmental and Modelling Group (EMG) and New and Emerging Respiratory Virus Threats Advisory Group (NERVTAG). SARS-COV-2: transmission routes and environments, 22 October 2020. <https://www.gov.uk/government/publications/sars-cov-2-transmission-routes-and-environments-22-october-2020>. Updated 2020. Accessed March 19, 2021.