World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

VACCINE USE TO COVID 19

[1*]Shubham P. Mankar, [*2]Vanshika D. Khemnar, [3]Vrushali B. Kuldharan and [4]Ambika S. Kakade

[1]Reserch Scholar- Assistant Professor Ashvin College of Pharmacy, Manchi Hill, Ashvi BK.
[2,3,4.]Reserch Scholar: B-Pharm. Ashvin College of Pharmacy, Manchi Hill, Ashvi BK.

Corresponding Author: Shubham P. Mankar & Vanshika D. Khemnar
Reserch Scholar- Assistant Professor Ashvin College of Pharmacy, Manchi Hill, Ashvi BK.
Reserch Scholar: B-Pharm. Ashvin College of Pharmacy, Manchi Hill, Ashvi BK.

Article Received on 30/08/2021 **Article Revised on 20/09/2021** **Article Accepted on 10/10/2021**

ABSTRACT

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is a new type of coronavirus that causes the Coronavirus Disease 2019 (COVID-19), which has been the most challenging pandemic in this century. Considering its high mortality and rapid spread, an effective vaccine is urgently needed to control this pandemic. As a result, the academia, industry, and government sectors are working tightly together to develop and test a variety of vaccines at an unprecedented pace. In this review, we outline the essential corona virus biological characteristics that are important for vaccine design. In addition, we summarize key takeaways from previous vaccination studies of Severe Acute Respiratory Syndrome Coronavirus (SARS- CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV), highlighting the pros and cons of each immunization strategy. Finally, based on these prior vaccination experiences, we discuss recent progress and potential challenges of COVID-19 vaccine development.

KEYWORDS: In this review, we outline the essential corona virus biological characteristics that are important for vaccine design.

INTRODUCTION

Corona viruses (CoVs) are a group of related viruses that can cause respiratory tract infection in humans ranging from mild symptoms to lethal outcomes. Until now, there are seven genera of CoVs that are known to infect humans.[1] Four of these genera, including Human Coronavirus 229E (HCoV-229E), Human Coronavirus OC43 (HCoV-OC43), Human Coronavirus NL63 (HCoV-NL63), and Human Coronavirus HKU1 (HCoV-HKU1), only cause relatively mild and self-limiting respiratory symptoms.[2] Alternatively, the other three CoVs, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), Middle East Respiratory Syndrome Coronavirus (MERS-CoV), and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), are highly pathogenic and can lead to severe respiratory diseases and fatal outcome in infected patients. The first lethal corona virus SARS-CoV emerged in 2002 in Guangdong Province, China. During the 2002–2004 outbreak, SARS-CoV had infected 8,098 people and resulted in 774 SARS-associated deaths (~ 10% mortality rate) across 29 countries before it disappeared.[3] In 2012, MERS-CoV emerged in Saudi Arabia. It caused two outbreaks in South Korea in 2015 and in Saudi Arabia in 2018, and still has ongoing reports of sporadic cases nowadays. As of January 2020, there are 2,519 confirmed MERS cases and 866 deaths (~ 35% mortality rate) across 27 countries.[4] In December 2019, a new type of CoV that can cause severe respiratory illness emerged in Wuhan, China. The World Health Organization named this novel virus SARS-CoV-2 and the disease COVID-19, or Coronavirus Disease 2019. The clinical manifestation of COVID-19 can vary from asymptomatic and mild flu-like symptoms to acute respiratory distress syndrome and death. Long-term pulmonary, cardiological,and neurological complications have also been reported in COVID-19 cases.[5] Compared with SARS-CoV and MERS-CoV, SARS-CoV-2 is highly contagious with an estimated reproductive number of 2.2 (one existing COVID-19 case can cause an average of 2.2 new infections).[6] In addition, its ability to spread through asymptomatic patients has posed a great challenge to containment measures.[7] By October 2020, SARS-CoV-2 has infected more than 43 million individuals and resulted in about 1.15 million deaths (~ 3% mortality rate) in 235 countries, areas or territories worldwide.[8] Needless to say, COVID-19 has become the most serious public health crisis of our generation and has a profound impact on the global economy and geopolitics. Although our understanding of pathogenic CoVs has been steadily accumulating for about two decades, no effective vaccine has yet been approved for the prevention of human CoV infection. Considering the rapid spread and high mortality of COVID-19, an effective vaccine is urgently needed to

control this pandemic. In this review, we summarize relevant CoV biology, SARS and MERS immunization strategies, and recent efforts of COVID-19 vaccine development. We hope this review can provide essential knowledge for any researcher who is interested in COVID-19 vaccine development.

Coronavirus biology and its implication on vaccine development

Coronaviruses, whose name derives from their characteristic crown-like appearance under the electron microscope, are enveloped RNA viruses with a diameter of approximately 80–160 nm. The genome of CoVs is a ~ 30 kb single-stranded positive-sense RNA molecule, which is the largest genome of all known RNA viruses. The 5′-terminus of the CoV genome contains two overlapping open reading frames (ORFs): ORF 1a and ORF 1b, spanning two-thirds of the genome length (Fig. 1a). ORF 1a and ORF 1ab can be translated into two polyproteins (pp), pp1a and pp1ab, which are further cleaved into 16 non-structural proteins (Nsps) involved in viral genome replication and subgenomic mRNA synthesis. The 3′-terminus of the CoV genome encodes four major structural proteins in the order of spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins (Fig. 1a). S, E, M protein forms the envelope of the CoV, while N protein forms the capsid to pack genomic RNA (Fig. 1b). The 3′-terminus of the genome also encodes multiple accessory proteins, which are usually genus-specific and can help CoV evade the immune system or increase virulence.[9,10,11] For instance, SARS-CoV contains accessory protein ORF 3a, 3b, 6, 7a, 7b, 8a, 8b and 9b, MERS-CoV contains ORF 3, 4a, 4b, 5, 8b, and SARS-CoV-2 contains ORF 3a, 6, 7a, 7b, 8, 10 (Fig. 1a).

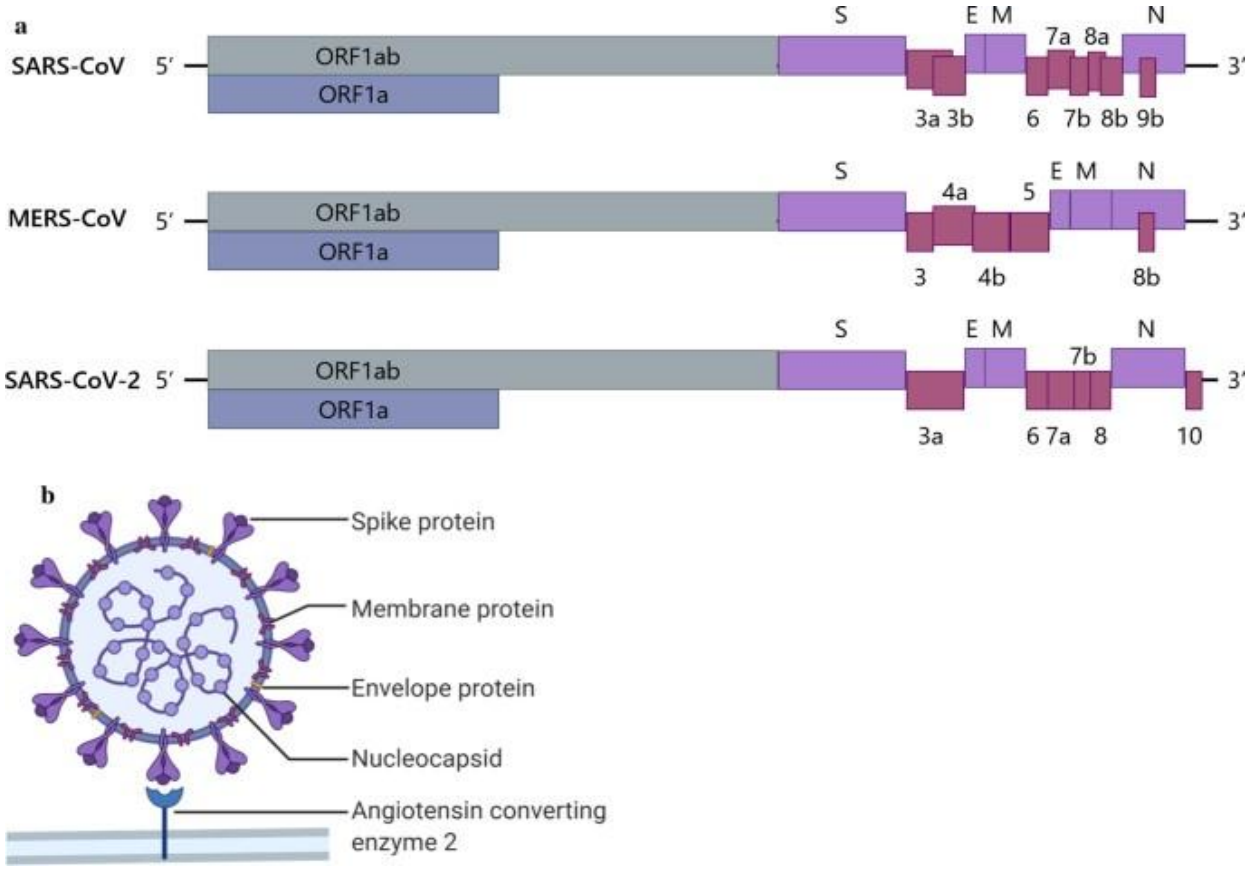

Fig. 1:

The genome and virion structure of coronaviruses (CoVs). **a** The genome structure of SARS-CoV, MERS-CoV, and SARS-CoV-2.[12,13,14] The 5′-terminus of the CoV genome contains two overlapping open reading frames (ORFs): ORF 1a and ORF 1b, spanning two-thirds of the genome length. ORF 1a and ORF 1ab can be translated into two polyproteins (pp), pp1a and pp1ab, which are further cleaved into 16 non-structural proteins (Nsps). The 3′- terminus of the CoV genome encodes four major structural proteins in the order of spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins. Genus-specific accessory proteins are also encoded at the 3′-terminus of the CoV genome. The virion structure of SARS-CoV-2. The spike (S), envelope (E), membrane (M) proteins form the envelope of the CoV, and the nucleocapsid (N) proteins form the capsid to pack the genomic RNA. The spike protein binds to angiotensin converting enzyme 2 (ACE2) on the cell membrane, which allows the virus to enter the cell. (Created with BioRender.com.)

Many viral proteins are essential for the life cycle of CoVs. For entering target cells, S protein first binds to cellular receptors through its receptor-binding domain (RBD), and the receptor-virus complex is subsequently translocated to endosomes (Fig. 2). Both SARS-CoV and SARS-CoV-2 S proteins bind to angiotensin-converting enzyme 2 (ACE2), while the S protein of MERS-CoV uses dipeptidyl peptidase-4 (DPP4) as its cellular receptor (Fig. 1b).[16] At the endosome, S protein is further cleaved into S1 (RBD-containing) and S2 (non-RBD-containing) subunits, and the S2 subunit mediates fusion between the viral envelope and the host cell membrane.[15] After entering the cell, several Nsps, particularly RNA-dependent RNA polymerase (Nsp12) and helicase (Nsp13), mediate the replication of the CoV genome and the transcription of CoV mRNA.[17] The CoV mRNA is further translated into different nonstructural and structural proteins. The N proteins bind to CoV genomic RNA to form viral nucleocapsids, and S, E, M proteins form the envelope of CoV. After assembly, viral particles bud through an endoplasmic reticulum (ER)-Golgi pathway and exit the cells by exocytosis(Fig. 2).[15]

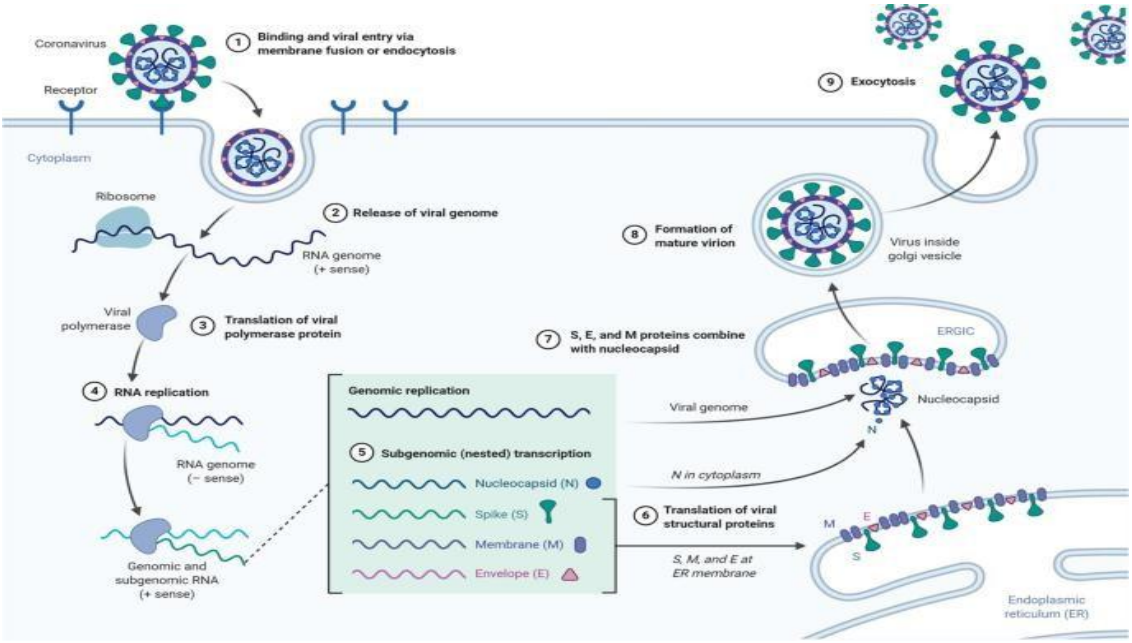

Fig. 2:

The life cycle of SARS-CoV-2.[9,10,15] Upon binding to the membrane receptor ACE2, SARS-CoV-2 virion enters the host cell and releases its plus-strand RNA genome. The plus- strand RNA translates pp1a and pp1ab, which are further cleaved into multiple non-structural proteins (Nsps) including an RNA-dependent RNA polymerase (Nsp12). The RNA-dependent RNA polymerase transcribes a negative-strand genomic RNA, and then uses this negative-strand genomic RNA as template to generate more plus-strand genomic RNA (genomic replication) and many different subgenomic RNAs (subgenomic transcription). The subgenomic RNAs are further translated into major structural proteins (N, S, M, E), which will assemble with plus-strand genomic RNA to form a mature virion in lumen of the ER. Finally, the whole virus leaves the cell through exocytosis.

The S protein is particularly important for virus-cell receptor binding and virus-cell membrane fusion, suggesting that it can be an effective target for CoV vaccine design. In fact, studies have shown that antibodies generated against the S protein are long-lasting and immunodominant in recovered SARS patients.[18,19] In addition, several studies have demonstrated that the anti-S antibody can neutralize SARS-CoV and MERS-CoV and provides protective effects in animals and humans.[20,21,22] Moreover, many S protein-based vaccines against SARS-CoV and MERS-CoV have been shown to elicit potent oborate that CoV S protein serves as an ideal vaccine target to induce neutralizing antibodies and protective immunity. Besides S protein, other structural proteins have also been tested as vaccine targets. N protein- based vaccines usually cannot induce neutralizing antibodies, likely due to the fact that N protein is not displayed on the CoV surface. However, N protein has the advantage oimmune responses and protective effects in preclinical models.[23,24,25,26,27] These results corrf being more conserved across CoV species than S protein, making it a potential target for a T-cell inducing, universal CoV vaccine.[16] One recent study has shown that a viral vector vaccine expressing N protein can induce CD4+ T cell-dependent protection against SARS-CoV and MERS-CoV,suggesting the feasibility of N protein-based T-cell inducing CoV vaccines.[28] M protein-based vaccines, on the other hand, can induce a high titer of antibody response in immunized animals.[29] However,

no neutralization antibody or protective immunity data of M protein-basedvaccines in preclinical models have been demonstrated. Finally, very few CoV E protein-based immunization studies have been reported so far, and none of the studies demonstrated induction of neutralizing antibodies or protective immunity.[30]

There are also immunopathological complications associated with the SARS-CoV and MERS-CoV vaccines that require addressing and further optimization. One adverse effect is the induction of antibody-dependent enhancement (ADE) effect, which is usually caused by vaccine- induced suboptimal antibodies that facilitates viral entry into host cells.[11,31] A study found that SARS-CoV vaccine based on full-length S protein enhances SARS-CoV infection of human cell lines in vitro. [32] Additionally, two studies have also shown that anti-S protein serum results in increased viral infectivity of SARS-CoV.[33,34] These results raise safety concerns for S protein-based SARS-CoV and MERS-CoV vaccines. One potential strategy to overcome the ADE problem is to design vaccines that only contain major neutralizing epitopes, such as the S1 subunit or the RBD domain of the S protein. This strategy can decrease the induction of non- neutralizing antibodies by CoV vaccines and therefore reduce the ADE effect. Another potential adverse effect is vaccine-induced eosinophilic immunopathology, which is an unwanted Th2- skewed immune response elicited by vaccination.[11,35] At least two studies have reported that whole inactivated virus vaccine of SARS-CoV induces eosinophilic proinflammatory pulmonary response after mice challenged with SARS-CoV. [36,37] In addition, one study also reported that immunization with SARS-CoV virus-like particle (VLP) vaccine leads to eosinophilic immunopathology in the lung after viral challenge. In order to prevent this Th2-type immunopathology, a few studies have worked on adjuvant optimization. They found that appropriate adjuvants, such as Toll-like receptor agonist and delta-inulin polysaccharide, can increase serum neutralizing antibody titers and reduce lung eosinophilic immunopathology.[38,39] Their results provide a promising strategy to deal with Th2-skewed immune response induced by some CoV vaccines.

Previous progress of SARS-CoV and MERS-CoV immunization strategies

Various forms of vaccines targeting SARS-CoV and MERS-CoV have been developed and tested in preclinical models. However, only a few of them entered clinical trials and none of them have been FDA approved. These approaches include protein subunit vaccines, virus-like particle vaccines, DNA vaccines, viral vector vaccines, whole-inactivated vaccines and live-attenuated vaccines. The following sections outline the principles of various forms of SARS-CoV and MERS-CoV vaccine development (Table 1), and the latest results from both preclinical studies and clinical trials (Table 2).

Table 1: Advantages and disadvantages of different vaccine platforms.

Vaccine platform	Advantages	Disadvantages	Clinically approved examples
Whole inactivated virus vaccine	Stronger immune response; Safer than live attenuatedvirus	Potential epitope alteration by inactivation process	Typhoid, Cholera, Hepatitis A virus, Plague, Rabies, Influenza, Polio (Salk)
Live attenuated virus vaccine	Stronger immune response; Preservation of native antigen; Mimicking naturalinfection	Risk of residual virulence, especially forimmunocompromised people	Measles, Mumps, Polio (Sabin), Rota virus, Yellow Fever, Bacillus Calmette–Guérin (BCG), Rubella, Varicella
Viral vectorvaccine	Stronger immune response; Preservation of native antigen; Mimicking naturalinfection	More complicated manufacturing process;Risk of genomic integration; Response dampened by pre- existing immunity against vector	Ebola virus
Subunit	Safe and well-	Lower immunogenicity;	Pertussis, Influenza, *Streptococcus*
Vaccine platform	**Advantages**	**Disadvantages**	**Clinically approved examples**
vaccine	tolerated	Requirement of adjuvant or conjugate toincrease immunogenicity	*pneumoniae, Haemophilus influenzaetype b*
Viral-likeparticle vaccine	Safe and well- tolerated; mimicking native virus conformation	Lower immunogenicity;More complicated manufacturing process	Hepatitis B virus, HumanPapillomavirus
DNA vaccine	Safe and well- tolerated; Stable under room temperature; Highly adaptable tonew pathogen; Native antigen expression	Lower immunogenicity;Difficult administrationroute; Risk of genomic integration	NA
RNA vaccine	Safe and well- tolerated; Highly adaptable to new pathogen; Native antigen expression	Lower immunogenicity;Requirement of low temperature storage and transportation; Potentialrisk of RNA-induced interferon response	NA

Table 2: Clinical trials of SARS, MERS and COVID-19 vaccines.

Platform	Vaccine	Group	Status	Ref
SARS Vaccine Clinical Trials				
Inactivatedvirus	Inactivated SARS-CoV vaccine (ISCV)	Sinovac	Phase I, completed	Lin et al. (2007) No NCT ID
DNA vaccine	VRC-SRSDNA015-00-VP	NIAID	Phase I, completed	Martin et al. (2008)NCT00099463
Platform	**Vaccine**	**Group**	**Status**	**Ref**
MERS Vaccine Clinical Trials				
DNA vaccine	GLS-5300 (INO-4700)	GeneOne Life Science /InovioPharmaceuticals/International Vaccine Institute	Phase I, completed	Modjarrad et al. (2019) NCT02670187
DNA vaccine	GLS-5300 (INO-4700)	GeneOne Life Science/Inovio Pharmaceuticals/ International Vaccine Institute	Phase I/IIa, completed	NCT03721718
Viral vector vaccine	MVA-MERS-S	CTC North GmbH & Co. KG	Phase I, completed	Koch et al. (2020)NCT03615911
Viral vector vaccine	MVA-MERS-S_DF1	CTC North GmbH & Co. KG	Phase Ib, not yetrecruiting	NCT04119440
Viral vector vaccine	ChAdOx1 MERS	University ofOxford	Phase I, recruiting	Folegatti et al. (2020) NCT03399578
Viral vector vaccine	ChAdOx1 MERS	King Abdullah International Medical ResearchCenter/University of Oxford	Phase I, recruiting	NCT04170829
Viral vector vaccine	BVRS-GamVac- Combi	Gamaleya Research Instituteof Epidemiology and Microbiology/Acellena Contract Drug Research and Development	Phase I/II, recruiting	NCT04128059

Platform	Vaccine	Group	Status	Ref	
Viral vector	BVRS-GamVac	Gamaleya	Phase I/II, recruiting	NCT04130594	
vaccine		Research Institute			
		of Epidemiology			
		and Microbiology			
COVID-19 Vaccine Clinical Trials					
Protein	NVX-CoV2373	SARS-CoV-2	Novavax	Phase III	Keech et al.
subunit		rS/Matrix-M1			(2020)
		Adjuvant			2020-004123-16
					NCT04533399
RNA	mRNA-1273	LNP-	Moderna/NIAID	Phase III	Jackson et al.
		encapsulated			(2020)
		mRNA			Anderson et al.
					(2020)
					NCT04470427
RNA	BNT162b1	LNP-mRNAs	BioNTech/Fosun	Phase III	Mulligan et al.
	BNT162b2		Pharma/Pfizer		(2020)
					Sahin et al.
					(2020)
					Walsh et al.
					(2020)
					NCT04368728
Viral vector	AZD1222	ChAdOx1-S	University of	Phase III	Folegatti et al.
			Oxford/AstraZeneca		(2020)NCT04516746

					NCT04540393
					ISRCTN8995142
					CTRI/2020/08/02
					7170

Platform	Vaccine	Group	Status	Ref	
Viral vector	Ad5-nCoV	Adenovirus Type5	CanSino Biological Inc./Beijing Institute ofBiotechnology	Phase III	Zhu et al.(2020) Zhu et al. (2020) NCT04526990 NCT04540419
Viral vector	Gam-COVID-Vac	Adeno-based (rAd26-S + rAd5-S)	Gamaleya ResearchInstitute	Phase III	Logunov et al.(2020) NCT04530396 NCT04564716
Viral vector	Ad26.COV2.S	Adeno-based	Janssen PharmaceuticalCompanies	Phase III	NCT04505722
Inactivated virus	Adsorbed COVID-19(inactivated) Vaccine	inactivated	Sinovac	Phase III	NCT04456595 NCT04582344 669/UN6.KEP/ EC/2020
Inactivated virus	Inactivated SARS-CoV-2 vaccine (Verocell)	Inactivated	Wuhan Institute of Biological Products/Sinopharm	Phase III	Xia et al. (2020) ChiCTR2000034780 ChiCTR2000039000
Inactivated virus	BBIBP-CorV	Inactivated	Beijing Institute of Biological Products/Sinopharm	Phase III	Xia et al. (2020) ChiCTR2000034780 NCT04560881
Protein subunit	Recombinant new coronavirus vaccine (CHO cell)	Adjuvanted recombinant RBD-Dimer	Anhui Zhifei Longcom Biopharmaceutical/Institute of Microbiology, Chinese Academy of Sciences	Phase II	NCT04466085
RNA	CVnCoV	mRNA	Curevac	Phase II	NCT04515147
Protein	KBP-COVID-19	S protein RBD-	Kentucky Bioprocessing,	Phase I/II	NCT04473690

Platform	Vaccine	Group	Status	Ref	
subunit		based	Inc		
Protein subunit	SARS-CoV-2 vaccine	Adjuvanted S protein	Sanofi Pasteur/GSK	Phase I/II	NCT04537208
RNA	ARCT-021	mRNA	Arcturus/Duke-NUS	Phase I/II	NCT04480957
DNA	INO-4800	DNA plasmid with electroporation	Inovio Pharmaceuticals /International Vaccine Institute	Phase I/II	NCT04447781NCT04336410
DNA	AG0301-COVID19	Adjuvanted DNAplasmid	OsakaUniversity/AnGes/Takar a Bio	Phase I/II	NCT04463472NCT04527081
DNA	nCov Vaccine	DNA plasmid	Cadila HealthcareLimited	Phase I/II	CTRI/2020/07/026352
DNA	GX-19	DNA Vaccine	Genexine Consortium	Phase I/II	NCT04445389
Inactivated	BBV152A BBV152B BBV152C	Inactivated	Bharat Biotech	Phase I/II	NCT04471519 CTRI/2020/09/027674
Inactivated	Inactivated SARS-CoV-2 Vaccine	Inactivated	Institute of Medical Biology, Chinese Academy of Medical Sciences	Phase I/II	NCT04470609
Inactivated	QazCovid-in	Inactivated	Research Institute forBiological Safety Problems, Rep of Kazakhstan	Phase I/II	NCT04530357
VLP	RBD SARS-CoV-2HBsAg VLP	RBD-HBsAg VLPs	SpyBiotech/Serum Institute of India	Phase I/II	ACTRN12620000817943
Protein	SCB-2019	Adjuvanted S	Clover	Phase I	NCT04405908

Platform	Vaccine	Group	Status	Ref	
subunit		protein	Biopharmaceuticals Inc./GSK/Dynavax		
Proteinsubunit	COVAX-19	S protein with Advax-SM adjuvant	Vaxine Pty Ltd/Medytox	Phase I	NCT04453852
Proteinsubunit	SARS-CoV-2 Sclamp vaccine	Molecular clamp stabilized S protein with MF59 adjuvant	University of Queensland/CSL/Seqirus	Phase I	ACTRN12620000674932p ISRCTN512329 65
Proteinsubunit	MVC-COV1901	S-2P protein + CpG1018	Medigen Vaccine Biologics Corporation/NIAID/Dynavax	Phase I	NCT04487210
Proteinsubunit	Soberana 01	S protein RBDwith Adjuvant	Instituto Finlay deVacunas, Cuba	Phase I	IFV/COR/04
Proteinsubunit	EpiVacCorona	Adjuvanted peptide antigen	FBRI SRC VBVECTOR, Rospotrebnadzor,Koltsovo	Phase I	NCT04527575
Proteinsubunit	Recombinant SARS-CoV-2 vaccine	S protein RBD(Sf9 cells)	West China Hospital, Sichuan University	Phase I	ChiCTR2000037518
Proteinsubunit	IMP (CoVac-1)	Multipeptide cocktail of SARS-CoV-2 HLA-DR peptides	University Hospital Tuebingen	Phase I	NCT04546841
Proteinsubunit	UB-612	S1-RBD-protein	COVAXX	Phase I	NCT04545749

Platform	Vaccine	Group	Status	Ref	
RNA	LNP-nCoVsaRNA	self-amplifying ribonucleic acid (saRNA) encoding S protein	Imperial College London	Phase I	ISRCTN17072692
RNA	SARS-CoV-2 mRNA vaccine	mRNA encodingS protein RBD	People's Liberation Army (PLA) Academyof Military Sciences/Walvax Biotech	Phase I	ChiCTR2000034112
Viral vector	hAd5-S-Fusion + N-ETSD vaccine	hAd5 Spike (S) + Nucleocapsid (N)	ImmunityBio, Inc. & NantKwest Inc	Phase I	NCT04591717
Viral vector	GRAd-COV2	Replication defective Simian Adenovirus (GRAd)	ReiThera/LEUKOCARE /Univercells	Phase I	NCT04528641
Viral vector	Ad5-nCoV	Ad5-based	CanSino Biological Inc/ Institute of Biotechnology, Academyof Military Medical Sciences, PLA of China	Phase I	NCT04552366
Viral vector	VXA-CoV2-1	dsRNA- adjuvanted Ad5	Vaxart	Phase I	NCT04563702
Viral vector	MVA-SARS-2-S	MVA + spikeprotein (S)	Ludwig-Maximilians - University of Munich	Phase I	NCT04569383
Viral vector	V590	VSV + S protein	Merck Sharp & Dohme/IAVI	Phase I	NCT04569786
Viral vector	TMV-083	Measles-vectorbased	Institute Pasteur/Themis /Univ. ofPittsburg CVR/ Merck Sharp & Dohme	Phase I	NCT04497298

Platform	Vaccine	Group	Status	Ref	
Viral vector	DelNS1-2019-nCoV-RBD-OPT1	Intranasal flu-based-RBD	Beijing Wantai Biological Pharmacy/XiamenUniversity	Phase I	ChiCTR2000037782
Inactivated	inactivated SARS-CoV-2 Vaccine	Inactivated	Beijing MinhaiBiotechnology	Phase I	ChiCTR2000038804
VLP	Recombinant Coronavirus-Like Particle COVID 19Vaccine	CpG 1018- or AS03-adjuvantedPlant-derived VLP	Medicago Inc	Phase I	NCT04450004

Protein subunit vaccine

Up to now, there have been 13 SARS-CoV-2 protein subunit vaccines entering clinical trials. Among these vaccines, a leading company Novavax, with its NVX-CoV2373 vaccine, has entered a phase IIb trial in South Africa (NCT04533399) and a phase III trial in the UK (2020- 004123-16). NVX-CoV2373 contains a prefusion stabilized full-length spike protein adjuvanted with their proprietary saponin-based adjuvant. In a preclinical trial, the vaccine induced neutralizing antibodies and prevented viral replication in the respiratory tract in macaques challenged with the virus. The vaccine also induced binding and neutralizing antibodies in all participants in the phase I trial. In their phase I trial, they also observed a dose sparing effect by the adjuvant. They found that both adjuvanted 5 ug and 25 ug dose regimens induced significantly high titers of neutralizing antibody compared to the placebo group and the 25 ug dose without adjuvant group. Another vaccine that has entered the phase II trial is Anhui Zhifei Longcom's recombinant new coronavirus vaccine (NCT04466085). Instead of using the full- length S protein, Anhui Zhifei Longcom's vaccine only contains the RBD of the SARS-CoV-2 S protein. However, no further design or data has been provided so far. For the other candidate SARS-CoV-2 protein subunit vaccines, most of them also utilize either full-length S protein or the RBD of S protein as their vaccine antigen. Notably, one recent study has described a generalizable strategy to enhance the immunogenicity of protein subunit coronavirus vaccines. They identified a disulfide-linked dimeric form of MERS-RBD that is significantly more immunogenic and protective than its conventional monomeric counterpart. They applied the same strategy to SARS-CoV-2 and has demonstrated a 10–100-fold enhancement of neutralizing antibody titers. Therefore, this framework of immunogen design could be universally applicable to all protein subunit coronavirus vaccines in the future.

DNA vaccine

There are 4 DNA vaccines for SARS-CoV-2 currently under clinical trials. Among these developers, Inovio is a leading company that has published results on MERS-CoV and SARS-CoV-2 DNA vaccines. Inovio's SARS-CoV-2 DNA vaccine INO-4800 encodes the full length S protein and is administered intradermally with a hand-held device CELLECTRA to electroporate the skin cell. Having experience in the phase I/IIa trial of their MERS vaccine (INO-4700), they are using the same platform for the SARS-CoV-2 vaccine INO-4800. They have demonstrated that the vaccine induces neutralizing antibodies and Th1-skewed immune responses in animal models including mice, guinea pigs, and rhesus macaques.[59] The vaccine is now in two phase I/II trial (NCT04447781 and NCT04336410). The interim analysis of the two phase I trials showed it induced humoral and T cell immune responses in 94% participants after two doses while only caused adverse events of grade 1 or below.

RNA vaccine

Although there were no RNA vaccine studies for SARS-CoV or MERS-CoV in the past two decades, there have already been 6 novel RNA vaccines reaching clinical trials for SARS- CoV-2 since the outbreak of COVID-19. RNA vaccines consist of viral antigen-encoding messenger RNAs that can be translated by human cells to produce antigenic proteins and stimulate the immune system. RNA vaccines are usually delivered in complex with additional agents, such as protamine or lipid- and polymer-based nanoparticles, to increase its efficacy. Similar to DNA vaccines, RNA vaccines have the advantages of being highly adaptable to new pathogens and being able to recapitulate the native conformation and modifications of antigenic proteins. Furthermore, compared with DNA vaccines, RNA vaccines have some additional benefits. Unlike DNA, RNA does not interact with host-cell DNA and therefore obviate the risks of genomic integration. Besides, RNA vaccines can be given through multiple routes including traditional intravenous injection, whereas DNA vaccines need to be administered via special devices like electroporation or gene gun. Nevertheless, RNA vaccines do have some drawbacks. Exogenous RNA can activate interferon-mediated antiviral immune response and lead to stalled translation and mRNA degradation, which suppress the efficacy of RNA vaccines. In addition, interferon signaling is associated with inflammation and potential autoimmunity. Even though there have not been severe cases of RNA vaccine-induced autoimmune diseases, it is important to carefully evaluate this potential adverse effect.

Moderna and BioNTech/Pfizer are the two leading developers for a SARS-CoV-2 RNA vaccine. Moderna's mRNA-1273 vaccine encodes a stabilized prefusion spike trimer, in which theysubstituted the amino acids at 986 and 987 with proline to stabilize the spike protein in its prefusion conformation. The nucleotides of the mRNA were also modified not only to increase its translation and half-life but also to prevent activation of interferon-associated genes upon entering the cell. The preliminary report for their phase I clinical trial showed that: (1) neutralizing antibodies were detected in all 45 patients after two doses of immunization; (2) antibody titers of immunized patients were higher than convalescent serum after two doses of vaccination; (3) Th1-biased immune responses were observed in immunized patients. There were some cases of systemic adverse events after the second dose of vaccination, but no grade 4 adverse events were observed. They concluded that 100 ug can induce a satisfactory immune response and thus will continue to use 100 ug dosage in phase III clinical trial (NCT04470427). In addition, they also expanded the same phase I trial to include 40 elderly participants with their age older than 55 years old. Their result demonstrated that 100 ug dose of mRNA-1273 induced higher binding- and neutralizing-antibody titers than the 25 ug dose, and the adverse events associated with mRNA-1273 were mild or moderate in these elderly

participants. On Nov 16, 2020, Moderna revealed the first interim analysis of their phase III trial (NCT04470427) . Their result showed that among 95 people who developed symptomatic COVID-19 after volunteering in this trial, only 5 of them were from the mRNA-1273 group, and the rest 90 cases were fromthe placebo group, resulting in a estimated vaccine efficacy of 94.5% . In addition, there were 11 volunteers who developed severe COVID-19 symptoms, and their analysis showed that all 11 cases were in the placebo group and none in the mRNA-1273 group. Their concurrent safety review also did not notice any significant safety concern. Therefore, their promising result suggested that the mRNA-1273 vaccine is safe and effective in preventing symptomatic COVID-19.

BioNTech and Pfizer's mRNA vaccine has four candidates, BNT162b1, BNT162b2, BNT162a1 and BNT162c2. BNT162b1 and BNT162b2 are both nucleoside modified mRNA (modRNA) vaccine. BNT162b1 encodes a trimerized RBD of spike protein while BNT162b2 encodes a full- length spike protein. On the other hand, BNT162a1 is a uridine mRNA (uRNA)-based vaccine and BNT162c2 is a self-amplifying mRNA (saRNA)-based vaccine. Up to now, BioNTech and Pfizer have published two BNT162b1 phase I/II trial results that were conducted in Germany (NCT04380701) and the US (NCT04368728), respectively. Both studies showed that the two-dose regimen of BNT162b1 elicited RBD-binding and neutralizing antibodies with titers above convalescent human serum. Analysis of cell-mediated immune responses showed Th1- skewed response in most participants, as demonstrated by the detection of IFNγ, IL-2 and IL-12 but not IL-4 in their assay. Although the German trial and the US trial used different dosages of vaccine, the two trials agreed with each other and showed that a regimen of 30–50 ug on day 1 and day 22 is able to elicit favorable immune response without severe adverse effects. Following these two papers, they also published another study comparing the vaccination responses between BNT162b1 and BNT162b2. BNT162b1 and BNT162b2 were shown to induce similar neutralizing titers in younger and older adults. However, BNT162b2 had less systemic reactogenicity in older adults. Therefore, they decided to move forward with BNT162b2 instead of BNT162b1 into a phase III clinical trial (NCT04368728). On Nov 18, 2020, Pfizer and BioNTech announced the efficacy analysis of their phase III clinical trial (NCT04368728) after meeting all primary efficacy endpoints. Their evaluation showed that BNT162b2 is 95% effective against COVID-19. This result was based on analyzing 170 confirmed COVID-19 cases, of which 162 cases of COVID-19 were observed in the placebo group while 8 cases in the BNT162b2 group . In addition, among 10 severe COVID-19 cases observed in this trial, 9 of them were in the placebo group and only 1 of them was in the BNT162b2 group. Notably, the observed efficacy in the elderly people was over 94%, which would help protect the most vulnerable population against COVID-19. No serious safety concern was observed among 43,000 enrolled participants. These data indicated BNT162b2 is another well-toleratedand efficacious COVID-19 vaccine.

Viral vector vaccine

Currently, there are 12 viral vector vaccines in clinical trials, and an additional 36 viral vector vaccines under preclinical development. Many viral vector platforms that have been tested in SARS-CoV and MERS-CoV are being explored in COVID-19 vaccines, including adenovirus (both human and non-human primates), measles virus, modified vaccinia virus Ankara (MVA), parainfluenza virus, rabies virus and vesicular stomatitis virus (VSV). Surprisingly, Venezuelan equine encephalitis (VEE) virus, which has been extensively studied in SARS and MERS vaccine, hasn't been tested in any COVID-19 vaccine studies yet. On the other hand, influenza virus vector, which hasn't been explored for SARS and MERS viral vector vaccines, are now gaining popularity for the development of COVID-19 viral vector vaccine. For COVID-19 viral vector vaccines that have entered clinical trials, 8 out of 12 are based on adenoviruses, and the four leading candidates in this platform are AZD1222 (or ChAdOx1 nCoV-19, developed by Astrazeneca and Oxford University), Gam-COVID-Vac (or Sputnik V, or rAd26S+rAd5- S, developed by Gamaleya Research Institute), Ad5 (developed by CanSino Biological Inc. and Beijing Institute of Biotechnology), and Ad26 (developed by Johnson & Johnson and Beth Israel Deaconess Medical Center).

AZD1222 is a chimpanzee adenovirus-based viral vector vaccine (ChAdOx1) expressing SARS- CoV-2 spike protein. This ChAdOx1 platform has been used to develop MERS-CoV vaccine, which has demonstrated promising preclinical and phase I clinical trial data. The AZD1222 vaccine team published their phase I/II trial interim report in July 2020 and showed that AZD1222 can elicit S protein-specific antibody and T-cell response and induce neutralizing antibody in all participants after the prime-boost regimen. No severe adverse effect has been observed. Based on this promising data, AZD1222 launched phase II/III trials in UK (2020- 001228-32) and phase III trials in Brazil (ISRCTN89951424), United States (NCT04516746), Russia (NCT04540393) and India (CTRI/2020/08/027170). In Sep 2020, the AZD1222 phase II/III trial in the UK was once put on hold for safety review because a participant has developed unexplained illness, but following later independent review in the UK determined that the trial is still safe and therefore the AZD1222 clinical trial resumed. On Nov 23, 2020, Astrazeneca annouced the interim analysis of their clinical trial in UK (2020-001228-32) and Brazil (ISRCTN89951424). Their pooled result showed that AZD1222 has an average efficacy of 70%, based on analyzing a total of 131 COVID-19 cases from 11,636 volunteers. Interestingly, one dose regimen showed 90% efficacy when AZD1222

was given as half first dose followed by a full second dose (n = 2,741) On the other hand, two full dose regimen had only 62% efficacy (n = 8,895). Due to the response discrepancy between different subgroups, additional trials may be needed to better determine the efficacy and the most suitable regimen of AZD1222. In addition, the Gam-COVID-Vac vaccine team has published their phase I/II trial results. They conducted two different trials, with one using frozen formulation (NCT04436471) and the other using lyophilized formulation (NCT04437875) of the vaccine. In both phase II trials, they tested their patients with heterologous prime-boost immunization of recombinant adenovirus type 26 vector encoding SARS-CoV-2 spike glycoprotein (rAd26-S) plus recombinant adenovirus type 5 vector encoding SARS-CoV-2 spike glycoprotein (rAd5-S). Their results showed that both frozen and lyophilized formulation of the vaccine induced potent neutralizing antibodies and CD4+ and CD8+ T-cell immune responses, with the immune response of frozen formulation being slightly stronger than the lyophilized formulation. Both vaccines were safe and well- tolerated in all participants. Now this vaccine is also entering phase III trial in Russia (NCT04530396) and Belarus (NCT04564716). On Nov 24, 2020, Gamaleya Research Institute announced the second interim analysis of Gam-COVID-Vac (or Sputnik V) phase III clinical trial (NCT04530396). Their result showed that Gam-COVID-Vac had a efficacy of 91.4% on Day 28 after the first dose, which was based on analyzing 39 confirmed cases among 18,794 volunteers. They also revealed that on Day 42 after the first dose (Day 21 after the second dose), the vaccine efficacy was even above 95%. There were no unexpected adverse effect documented during the trial]. These promising results suggested that Gam-COVID-Vac is safe and effective in preventing COVID-19. Furthermore, the Ad5 vaccine team, whose vaccine is based on human adenovirus 5, has also published their clinical data. In their phase II study, Ad5-vectored COVID-19 vaccine induces significant neutralizing antibodies and T-cell mediated immune response after single immunization. They tested two dosage, 1×10^{11} and 5×10^{10} viral particles, and showed that the 5×10^{10} dose causes less severe adverse reactions without compromising theimmunogenicity. Now this vaccine has advance to two phase III global multi-centered clinical trials (NCT04526990 and NCT04540419). Finally, Johnson & Johnson's Ad26-based COVID- 19 vaccine has also entered phase III clinical trial (NCT04505722), but no data from its earlier trial has been reported yet.

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