REVIEW ON CLINICAL MANIFESTATIONS OF COVID-19 VIRUS AND THE POSSIBLE ADAPTIVE IMMUNE RESPONSES

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Abstract:

Coronavirus disease 2019 (COVID-19), the highly contagious infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The disease has shown to put an enormous burden on the society and is a challenge to the physician owing to its infectivity, complications, morbidity and absence of proper medication. As medicine is an ever-changing science new research and clinical experience broaden our knowledge and bring changes in drug therapy. COVID-19 is now spreading throughout the world because of its high infectivity and communicability. Also there is a lack in the proper management of the disease. Because there is no definite and specific treatment for patients with COVID-19, some antiviral agents are prescribed to the patients, depending on their condition and location. It is widely accepted that the world will not return to its prepandemic normalcy until safe and effective vaccines become available and a global vaccination programme is successfully implemented. As COVID-19 is new to humankind and the nature of protective immune responses is poorly understood, it is unclear which vaccine strategies will be most successful. This review attempts to educate the readers about the molecular and structural complexity of COVID-19 virus; its clinical manifestations in humans; immune responses and a special reference on the various adaptive immune responsive strategies including vaccines and its limitations.

Keywords: COVID-19, Vaccine, Spike Protein, Vector, Immune response, Pandemic, SARS-CoV2

Review Methodology:

The literature search for the review was performed using few electronic databases: EMBASE, PubMed, Science Direct and Google Scholar. Many search keywords were used to conduct the literature search included the following: "COVID-19 review," Aetiology, host Immune responses in COVID Infection and "Coronavirus." Only full length English language review and full length research paper was taken for review purpose. Attempts were made to identify all literature related to COVID-19. No time limit was set for the search. Relevant titles and abstracts of the retrieved research papers and reviews were screened and appropriate and relevant research papers were taken into consideration. The following current research

topics like COVID aetiology, SARS COV pathogenesis, mode of transmission of nCOV, Molecular and Serological diagnosis methods and therapeutic approaches, Strategies in developing adaptive immune responses using vaccine molecules; and early supportive therapy and monitoring. Data were also made available from the notifications, articles, blogs published by WHO interim guidelines and from the Centre for Disease Control and Prevention (CDC).

Virology

As per the explanation released by World Health Organization (WHO) in December 2019, Patients presenting with viral pneumonia cases of unknown aetiology were identified in the city of Wuhan in central China. A novel coronavirus was subsequently identified as the causative pathogen,

provisionally named 2019 novel coronavirus (2019-nCoV). On February 11th 2020, WHO announced the rapidly spreading coronavirus disease as COVID-19. The coronavirus disease 2019 (COVID-19) become pandemic and emerging as a terrific threat globally. Coronavirus disease 2019 SARS-CoV-2 (COVID-19) is a zoonotic virus causing a variety of severe respiratory diseases. SARS-CoV-2 is closest to SARS-CoV and MERS-CoV family of viruses which share almost similar structure. Coronaviruses belong to the family Coronaviridae in the order Nidovirales. They are classified into four genera: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus. Among them, alpha- and betacoronaviruses infect mammals, gammacoronaviruses infect avian species, and deltacoronaviruses infect both mammalian and avian species. Representative alphacoronaviruses include human coronavirus NL63 (HCoV-NL63), porcine transmissible gastroenteritis coronavirus (TGEV), PEDV, and porcine respiratory coronavirus (PRCV) (Chan et al., 2013). Representative betacoronaviruses include SARS-CoV, MERS-CoV, bat coronavirus HKU4, mouse hepatitis coronavirus (MHV), bovine coronavirus (BCoV), and human coronavirus OC43. Representative gamma- and deltacoronaviruses include avian infectious bronchitis coronavirus (IBV) and porcine deltacoronavirus (PdCV), respectively. It is believed that the high prevalence of COVID-19 is a result of a lack of symptoms at onset in most of the patients (Dawood, 2020). Comparison of the lipid rafts of coronaviruses has indicated that the new strain COVID-19 has 80% identity with severe acute respiratory syndrome coronavirus (SARS-CoV).

Structural features of COVID-19 & Mechanism of host entry

Structural analysis shows that COVID-19 probably derives from a bat SARS-like coronavirus, which has mutated in the spike glycoprotein (protein S) and nucleocapsid N protein (Wu et al., 2020). Coronaviruses are large, enveloped, positive-stranded RNA viruses. They have the largest genome among all RNA viruses,

typically ranging from 27 to 32 kb. The genome is packed inside a helical capsid formed by the nucleocapsid protein (N) and further surrounded by an envelope. The COVID-19 complete genome was annotated to possess 14 open reading frames (ORFs) encoding 27 proteins. Sequence analysis revealed that COVID-19 has > 80% identity with SARS-CoV and 50% with MERS-CoV, which originated in bats (Guo et al., 2020). SARS-CoV-2 is a single-stranded positive-strand RNA virus whose genome encodes four structural proteins: spike (S), small protein (E), matrix (M), and nucleocapsid (N) (Chan et al., 2020). Among these, a frameshift between ORF1a and ORF1b guides the production of both pp1a and pp1ab polypeptides that are processed by virally encoded chymotrypsin-like protease (3CLpro) or main protease (Mpro), as well as one or two papain-like proteases for producing 16 with known or predicted RNA synthesis and modification functions non-structural proteins (NSPs 1-16). Besides ORF1a and ORF1b, other ORFs encode structural proteins, including spike, membrane, envelope, and nucleocapsid proteins and accessory proteic chains (Chan et al., 2020). Some coronaviruses also encode an envelope-associated hemagglutinin-esterase protein (HE). Different CoVs possess unique structural and accessory proteins translated by dedicated sgRNAs. The pathogenesis of CoVs and SARS-CoV-2 is related to the function of the NSPs and structural proteins. For example, researchers have outlined the role of NSPs in blocking the host's innate immune response (Lei et al., 2018). Among functions of structural proteins, the envelope has a crucial role in virus pathogenicity as it promotes viral assembly and release. Whereas the spike structural protein forms large protrusions from the virus surface, giving coronaviruses the appearance of having crowns (Fang Li 2016). The spike glycoproteins composed of two subunits (S1 and S2). The spherical external spike protein displays a characteristic crown shape with electron microscopy (Kim et al., 2020). Homotrimers of S proteins compose the spikes on the viral surface, guiding the link to host receptors (Song et al., 2018) that mediates coronavirus entry into host

cells. S protein of SARS-CoV is involved in receptor recognition, as well as virus attachment and entry, it represents one of the most important targets for the development of SARS vaccines and therapeutics (Du et al., 2009). The S protein is a type I fusion protein that forms trimers on the surface of the virion. It is composed of two subunits, with S1 responsible for receptor binding and S2 for membrane fusion (Walls et al., 2020; Wrapp et al., 2020). The SARS-CoV-2 utilizes angiotensin-converting enzyme 2 (ACE2) as the receptor for entry into target cells (Letko et al., 2020). Lipid molecules such as caveolins, clathrins and dynamin have a fundamental role in the internalization of viruses. These molecules are involved in the entry of viruses into host cells, and targeting host lipids is being studied as an antiviral strategy and could have various applications (Baglivo et al., 2020). On entry, SARS-CoV-2 interacts with the angiotensin-converting enzyme 2 (ACE2) receptor on bronchial and alveolar epithelial cells through its spike (S) protein receptor-binding domain (RBD), which is subsequently primed by a specific cellular serine protease, transmembrane protease serine 2 (TMPRSS2), to gain entry. The replication of the viral RNA is initiated with the synthesis of polyprotein 1a/1ab (pp1a/pp1ab). The transcription occurs through the replication-transcription complex (RCT) organized in double-membrane vesicles and via the synthesis of subgenomic RNAs (sgRNAs) sequences. Conversely, transcription termination occurs at transcription regulatory sequences, located between the so-called open reading frames (ORFs) that work as templates for the production of subgenomic mRNAs. The first ORF (ORF1a/b) translates two polyproteins, pp1a and pp1ab, and encodes 16 non-structural proteins (NSP); this takes up two-thirds of the viral RNA. The remaining ORFs encode structural proteins including spike (S) glycoprotein, small envelope (E) protein, matrix (M) protein and nucleocapsid (N) protein. Analysis of transcripts encoding ACE2 and TMPRSS2 by single-cell RNA sequencing has shown that these transcripts are co-expressed in various cell types (Mangalakumari et al., 2020). Therefore, the S

protein determines the infectivity of the virus and its transmissibility in the host (Hulswit et al., 2016). As this S protein is the major antigen inducing protective immune responses (Du et al., 2009; He et al., 2004; Li, 2016; Walls et al., 2020), all vaccines under development are directed against it. Clearly, it is pivotal to closely monitor antigenic evolution of the spike in the circulating viruses. As it is a heavily glycosylated protein, investigation of the effects of the site-specific glycans on infectivity and immune escape is also of unquestionable importance. RNA viruses are known to have higher mutation rates than DNA viruses (Duffy, 2018; Lauring and Andino, 2010). Amino acid changes in the surface protein can significantly alter viral function and/or interactions with neutralizing antibodies (Tsetsarkin et al., 2007). Although SARS-CoV-2 was only discovered in humans recently, mutations in the gene encoding Spike (S) protein are being continuously reported (Korber et al., 2020; Saha et al., 2020; Sheikh et al., 2020). Presumably, this change may have induced a conformational change in the S protein, thereby resulting in the increased infectivity. However, it remains largely unclear as to whether these reported variants could influence viral infectivity, transmissibility, or reactivity with neutralizing antibodies (Becerra Flores and Cardozo, 2020).

COVID-19 Mutants

Multiple variants of the SARS-COV-2 virus have been circulating in and around the world. According to the Centre for Disease Control and Prevention (CDC), three classifications of COVID-19 variants are being monitored, namely Variant of Interest (VOI), Variant of Concern (VOC), and Variant of High Consequence (VOHC). B.1.1.7, also known as the UK variant, was found in the south-east of England and is currently identified as a Variant of Concern (VOC). Experts suggest that this variant was 40-70% more infectious than other variants and raises death risks to 60%. The Brazil variant, scientifically known as P.1, is believed to be more contagious and dangerous than the previous mutation. E484K, an escape mutation allows the

variant to evade the antibodies. B.1.351, the South African variant was found in at least 20 countries, including the United Kingdom. Akin to the Brazil variant, E484K mutation allows this variant to dodge antibodies. Additionally, N501 mutation makes it more contagious. The Indian origin double mutant virus variant, scientifically termed as B.1.617, was first identified around March end in the state of Maharashtra and continues to drive India's second wave of coronavirus. It contains E484Q and L452R mutations, which makes it more infectious and enables it to escape antibodies. Additionally, recent reports suggest that a 'Triple mutation' COVID variant has been identified as B.1.618 in parts of West Bengal, Delhi and Maharashtra states in India.

Clinical manifestation and symptoms

COVID-19 possesses accessory proteins that interfere with the host's innate immune response (Guo et al., 2020). The causative agent of COVID-19, SARS-CoV-2 causes respiratory tract infection that can progress to severe acute respiratory syndrome and even multiple organ failure (Lv et al., 2020; Yang et al., 2020). The incubation period of the virus may vary with age and immune status. In general, it has been assumed that the incubation period is between 2 and 14 days, although cases have been observed up to 23 days after exposure. The main symptoms are easily seen in those aged over 70 years and in immunocompromised and diabetic individuals. Symptoms start with fever, dry cough and dyspnoea, as well as sore throat, nasal congestion, malaise; bilateral infiltrates may be seen on chest X-ray. However some cases are detected in the absence of fever. Clinical features of COVID-19 include the targeting of the lower airway, as well as upper respiratory tract symptoms like rhinorrhoea, sneezing, and sore throat, developed into gastrointestinal symptoms like diarrhoea (Rothen et al., 2020). Severe cases may present with sepsis, heart attack or even shock. Conversely, some cases may show mild illness or be asymptomatic. From WHO records, the period from symptom onset to death ranges from 6 to 41 days with a median of 14 days. This period

depends on the age and immune status of the individual and is shorter in those <70 years old (Koenig et al., 2020). Asymptomatic infections have been well documented and estimated that 33 percent of people with SARS-CoV-2 infection never develop symptoms. Patients with asymptomatic infection may have objective clinical abnormalities or atypical imaging abnormalities like evidence of typical ground-glass opacities or patchy shadowing on chest computed tomography (CT) examination. The SARS-CoV-2 utilizes angiotensin-converting enzyme 2 (ACE2) as the receptor for entry into target cells which negatively regulates the Renin-Angiotensin System (RAS) and plays a significant role in neurohumoral regulation of the cardiovascular system. When SARS-CoV-2 binds to ACE2, it alters the ACE2 signaling pathways, potentially causing acute myocardial and lung injury (Xiong et al., 2020). ACE2 receptors are abundantly distributed in the epithelia of the lung—especially the alveolar Type II (ATII) cells. Once the virus binds to the receptor, it spreads widely on entering the blood circulation. ACE2 receptors are also said to be present in the liver, digestive organs and kidneys. These tissues and organs could thus be potential targets for SARS-CoV-2 invasion (Fan et al., 2020). This explains why many patients with COVID-19 present with extra-pulmonary symptoms (Huang et al., 2020). When the over activated immune system is engaged in killing the virus, there is a sharp spike in the production of inflammatory factors, such as IL-2, IL-6, GCSF, MCP-1, TNF α , etc. This, in turn, may pave the way for a cytokine storm (Huang et al., 2020) and eventually lead to acute respiratory distress syndrome, secondary infections and multi-organ damage, resulting in death. Severe illness can occur in otherwise healthy individuals of any age, but it predominantly occurs in adults with advanced age or certain underlying medical comorbidities like Cardiovascular diseases, Diabetes mellitus, Chronic obstructive pulmonary disease and other lung diseases, Cancer (in particular hematologic malignancies, lung cancer, and metastatic disease), Chronic kidney disease, Solid organ or hematopoietic stem cell transplantation, Obesity, Smoking. Specific

demographic features and laboratory abnormalities have also been associated with severe disease. Such severe covid cases are characterized by markedly increased numbers of inflammatory monocytes and neutrophils in blood and CD14+CD16+ monocyte-derived macrophages in the airway and increased systemic levels of inflammatory cytokines and chemokines and from autopsy studies SARS-CoV-2 can be detected in multiple organs, including the lungs, pharynx, heart, liver, brain and kidneys (Mangalakumari et al., 2020).

Host Immunological Responses

Infection with SARS-CoV can trigger a series of humoral and cellular immune responses. Specific antibodies against SARS-CoV (immunoglobulin G (IgG) and IgM) were detectable approximately 2 weeks post-infection, reaching a peak 60 days post-infection and remaining at high levels until 180 days post-infection (Mo et al., 2005). High titres of neutralizing antibodies and SARS-CoV-specific cytotoxic T lymphocyte responses were detected in patients who had recovered from SARS (Xu et al., 2004), and the levels of the responses correlated well with the disease outcome (Li et al., 2006). This suggests that both humoral and cellular immune responses are crucial for the clearance of infection by SARS-CoV. Neutralizing antibodies and/or T-cell immune responses can be raised directly against several SARS-CoV proteins, but mainly target the S protein, suggesting that S protein-induced specific immune responses play important parts in the fight against SARS-CoV infection (Xu et al., 2004). The enzyme furin is present in the host cell and plays a vital role for the virus to enter, which was absent in SARS-CoV (Walls et al., 2020). Next, the virus starts to propagate with limited innate immune response and can be detected by nasal swabs. The virus then propagates and reaches the respiratory tract, where it faces a more robust innate immune response. At this stage, the disease is clinically manifest and an innate response cytokine may be predictive of the subsequent clinical course (Tang et al., 2005). The disease will be mild for 80% of the infected patients and mostly restricted to the upper and

conducting airways (Wu et al., 2020). With conservative symptomatic therapy, these individuals may be monitored and monitored at home. Approximately 20% of the infected patients develop pulmonary infiltrates and some of these develop very severe disease (Mason et al., 2020). The mortality rate of severe patients with COVID-19 can be as high as 49%, based on a recent epidemiological by China CDC (Wang et al., 2020). After being affected by virus immune responses to mediate antibody. The B cells are assisted by T cells to differentiate into plasma cells, which then produce antibodies specific to a viral antigen. A neutralizing nature antibody is efficient in fully blocking the virus from entering into host cells to limit the infection and plays a very intense protective role at the later stage of infection and prevents relapse of infection. IgM and IgG antibodies to SARS-CoV-2 are detectable within 1–2 weeks after the onset of symptoms in most infected individuals. Recent studies indicate that the magnitude of neutralizing antibody responses is positively correlated with disease severity. Thus the magnitude of the neutralizing antibody response in asymptomatic individuals is not only smaller but also decreases faster than in symptomatic individuals (Long et al., 2020). By contrast, a cellular immunity response can be observed inside the infected cells, which is mediated by T-lymphocytes. The overall adaptive immune response is directed by helper T cells, and cytotoxic T cells play a vital role in the clearance and cleaning of viral-infected cells (Kumar et al., 2020). Emerging evidence suggests the requirement of both antibody-mediated and T cell-mediated immunity for effective protection against SARS-CoV-2 (Sariol et al., 2020). One study found that among people who had recovered from COVID-19, 100% had S protein-specific CD4+ T cells in the circulation and 70% had S protein-specific CD8+ T cells in the circulation (Grifoni et al., 2020), and preclinical studies show a protective role of T cells in host defence against SARS-CoV (Zhao et al., 2010).

Current Therapeutic Approaches:

Although physical-distancing and other transmission-mitigation strategies implemented in

most countries during the current pandemic have prevented most citizens from being infected, these strategies will paradoxically leave them without immunity to SARS-CoV-2 and thus susceptible to additional waves of infection. Health-care workers, seniors and those with underlying health conditions are at particularly high risk (Flaxman 2020) (Sanche 2020). Because there is no definite and specific treatment for patients with COVID-19, some antiviral agents are prescribed to the patients, depending on their condition and location. Among the antiviral agents, remdesivir a phosphoramidate prodrug of an adenosine C-nucleoside and a broad spectrum antiviral agent is using widely for the COVID treatment (Siegel et al., 2017). Hydroxychloroquine and chloroquine are other immunosuppressant drugs that have a long history of clinical use for the treatment of malaria erythematosus and rheumatoid arthritis (Rynes 1997). Other antiviral drugs like Lopinavir, a protease inhibitor (Bin et al., 2020), Umifenovir (Boriskin et al., 2008), Favipiravir (Furuta et al., 2017), Oseltamivir a neuraminidase targeting drug (McClellan and Perry 2001) are widely being used for the COVID-19 treatment. Alternative medications are also emerging as a therapeutic solution for the control of COVID-19. A Randomized controlled Single blinded prospective multi centre clinical trial to investigate the safety and efficacy of ZingiVir-H, an ayurvedic molecule as an adjuvant therapy in hospitalized adults diagnosed with coronavirus disease 2019 (COVID-19) has shown promising recovery among Zingivir-H treated patients along with standard of care in a time of 4 days when compared with placebo treated group along with standard of care (https://ayurcentralonline.com/en/blogs/544_ZINGIVIR-H-FROM-PANKAJAKASTHURI-MIGHT-HELP-TR.html).

Vaccine as Adaptive Immune Response

It is widely accepted that the world will not return to its prepandemic normalcy until safe and effective vaccines become available and a global vaccination programme is successfully implemented. As COVID-19 is new to humankind and the nature of protective immune

responses is poorly understood, it is unclear which vaccine strategies will be most successful. Therefore, it is imperative to develop various vaccine platforms and strategies in parallel. Indeed, since the outbreak began, researchers around the world have been racing to develop COVID-19 vaccines, with at least 166 vaccine candidates currently in preclinical and clinical development (WHO report 2020). Vaccine design concerns the selection of antigens, vaccine platforms, and vaccination routes and regimen. The choice of vaccine platform determines the relative immunogenic strength of vaccine-derived viral antigens, whether an immune adjuvant is required and the nature of protective immunity. These attributes also determine the suitability of a vaccine for a particular route of vaccination, and whether a prime–boost vaccination regimen is required to increase vaccine-mediated protective immunity and its durability. Furthermore, the selection of live attenuated viral vaccines or a respiratory mucosal route of vaccination will require more stringent safety testing. The six major types of candidate vaccine for coronavirus disease 2019 (COVID-19) are in development phase or in clinical use (live attenuated virus, recombinant viral vectored, inactivated virus, protein subunit, virus-like particles and nucleic acid based), showing the number of candidate vaccines that are currently under clinical and preclinical development. The nucleic acid-based platform includes both mRNA vaccines (6 clinical and 16 preclinical) and plasmid DNA vaccines (4 clinical and 11 preclinical) (WHO report 2020). Interestingly a self-administered nitric oxide nasal spray (NONS) made by Vancouver-based biotech firm SaNOTize has been found to dramatically reduce Covid-19 viral load in infected patients after completing early-stage clinical trials in Canada and, most recently, the UK. SaNOTize, alongside Ashford and St Peter's Hospitals NHS Foundation Trust and Berkshire and Surrey Pathology Services in the UK, have announced results of Phase II trials indicating that SaNOTize's nasal spray represents a safe and powerful antiviral treatment that could prevent the transmission of Covid-19, shorten its duration, and reduce the severity of symptoms in those

already infected. In a randomised, double-blind, placebo-controlled Phase II trial that evaluated 79 confirmed cases of Covid-19, SaNOTize's early treatment significantly decreased the level of SARS-CoV-2, including in patients with high viral loads infected by the concerning UK variant of Covid-19. These recent advancement brings hope in the future vaccine advancements.

As of now, there are 29 vaccine candidates for COVID-19 in clinical evaluation and 139 vaccines in preclinical development. Clinical trials for many of the vaccines candidates are currently ongoing parallel and recruiting volunteers, and a couple of other candidates are

also about to enter clinical trials. Few Vaccines candidates are rolled out for patient usage in many countries under the government clearance as emergency usage. Preclinical evaluation of candidate vaccines requires the use of relevant animal models of COVID-19. Conventionally, the safety, immunogenicity and protective efficacy of experimental vaccines are rigorously evaluated and established in animal models first before clinical trials are begun. In the case of pandemic vaccine development, however, the preclinical and clinical stages of vaccine development are compressed and move forwards in parallel.

Table 1: List of Vaccine molecule in development and / or Emergency Usage Phase

S.No	Lead Molecule / Vaccine	Technology	Innovator	Immunization methods
1	ChAdOx1 nCov-19 (AZD-1222) / COVISHEILD	ChAd-vectored, non-replicating	University of Oxford, AstraZeneca	Expressing S protein; single dose or two repeated doses of IM injection
2	Ad5-nCoV	Ad5-vectored, non-replicating	CanSino Biologics Inc., Beijing Institute of Biotechnology	Expressing S protein; single dose of IM injection
3	mRNA-1273	Lipid nanoparticle-mRNA	Moderna, NIAID	Expressing S protein; two repeated doses of IM injection
4	PiCoVacc	Inactivated SARS-CoV-2	Sinovac Biotech	Multiple viral antigens; two repeated doses of IM injection
5	NVX-CoV2373	Protein subunit	Novavax	Recombinant S protein; two repeated doses of IM injection
6	BNT162b1	Lipid nanoparticle-mRNA	BioNTech, Pfizer, Fosun Pharma	RBD of S protein; two repeated doses of IM injection
7	BBIBP-CorV	Inactivated SARS-CoV-2	Sinopharm, Beijing Institute of Biological Products Co. Ltd	Multiple viral antigens; two repeated doses of IM injection
8	COVID-19 vaccine	Inactivated SARS-CoV-2	Sinopharm, Wuhan Institute of Biological Products Co. Ltd	Multiple viral antigens; two repeated doses of IM injection

9	INO-4800	Plasmid DNA	Inovio Pharmaceuticals, International Vaccine Institute	Expressing S protein; two repeated doses of intradermal injection plus electroporation
10	LNP-nCoVsaRNA	Lipid nanoparticle-saRNA	Imperial College London, Morningside Ventures	Expressing S protein; two repeated doses of IM injection
11	COVID-19 vaccine	Inactivated SARS-CoV-2	Chinese Academy of Medical Sciences	Multiple viral antigens; two repeated doses of IM injection
12	CVnCoV	Lipid nanoparticle-mRNA	CureVac	Expressing S protein; two repeated doses of IM injection
13	Gam-COVID-Vac Lyo	Ad5- or Ad26-vectored, non-replicating	Gameleya Research Institute	Single dose and heterologous Ad26 prime-Ad5 boost doses of IM injection
14	GX-19	Plasmid DNA	Genexine Consortium	Expressing S protein; two repeated doses of IM injection
15	SCB-2019	Protein subunit	Clover Pharmaceuticals, GlaxoSmithKline, Dynavax	Trimeric S protein; two repeated doses of IM injection
16	COVID-19 vaccine	Protein subunit	Anhui Zhifei Longcom Biologic Pharmacy, Chinese Academy of Medical Sciences	Dimeric RBD; two or three repeated doses of IM injection
17	ARCoV	mRNA	Academy of Military Medical Sciences, Walvax Biotechnology, Suzhou Abogen Biosciences	Expressing S protein; two repeated doses of IM injection?
18	COVID-19 vaccine	Plasmid DNA	AnGes Inc., Osaka University, Takara Bio	Expressing S protein; two repeated doses of IM injection
19	COVID-19 vaccine	Virus-like particle	Medicago, Laval University	Multiple viral antigens; two repeated doses of IM injection
20	Lunar-COV19	Self-replicating mRNA	Arcturus Therapeutics, Duke-National University of Singapore	Expressing S protein; one dose of IM injection

21	Covaxin	Inactivated SARS-CoV-2	Bharat Biotech, Indian Council of Medical Research, National Institute of Virology	Multiple viral antigens; two repeated doses of IM injection
22	ZyCov-D	Plasmid DNA	Zyklus Cadila	Expressing S protein; three repeated doses of intradermal injection
23	COVID-19 vaccine	Protein subunit	University of Queensland	Molecular clamp-stabilized S protein; two repeated doses of IM injection
24	Ad26.COV2-S	Ad26-vectored, non-replicating	Johnson & Johnson	Expressing S protein; two repeated doses of IM injection
25	KBP-COVID-19	Protein subunit	Kentucky Bioprocessing Inc.	Recombinant RBD-based protein; two repeated doses of IM injection
26	COVID-19 vaccine	VSV-vectored, replicating	Merck, IAVI	Expressing S protein; IM injection
27	COVAX19	Protein subunit	Vaxine Pty Ltd, Medytox, Central Adelaide Local Health Network	Recombinant S protein with Advax-SM adjuvant; single escalating dose of IM injection
28	MVC-COV1901	Protein subunit	Medigen Vaccine Biologics, Dynavax	Recombinant S protein; two repeated doses of IM injection
29	COVID-19 vaccine	Plasmid DNA	Entos Pharmaceuticals	Expressing S protein, IM injection

Live attenuated viral vaccines

Historically, several successful human vaccines, such as measles vaccine and the bacillus Calmette–Guérin (BCG) vaccine for tuberculosis (TB), have been based on attenuated strains of the actual pathogen (Plotkin et al., 2014), with loss or mutation of virulence genes through in vitro passage. It is now possible to rationally design attenuated virus strains by mutating or deleting virulence genes. These deletion mutants can often replicate to a limited extent in host cells but lose the ability to cause disease in vivo. Coronaviruses have several genes that are not required for replication and that can be deleted, leading to attenuation in vivo. Deletion of various non-structural proteins, as well as of the structural E protein, has been used as a strategy to engineer vaccine strains of several zoonotic and veterinary coronaviruses (Almazán et al., 2013) (Hou et al.,

2018). Deletion of the E protein leads to attenuation and generation of an efficacious vaccine strain (Hou et al., 2018), but reversion of the attenuated phenotype has been reported. Deletion of virulence factors may therefore provide a preferred mechanism of attenuation. For example, deletion of the 2'-O-methylase gene from the SARS-CoV genome removes the ability of the virus to hide its RNA from the host cell proteins MDA5 (also known as IFIH1) and IFIT1, thereby inducing a robust antiviral response in vivo. Another approach to viral attenuation is known as codon deoptimization, whereby the nucleic acid sequence is modified to use suboptimal codons to encode the wild-type amino acid sequence, which considerably slows the translation of the viral protein during infection. This approach can yield a virus that is highly attenuated in vivo but still able to replicate in

vitro if the correct viral protein is selected for deoptimization (Cheng 2015).

However, the generation of an attenuated strain of a pathogen for use as a vaccine requires demonstration of its inability to revert genetically to become pathogenic. This is particularly challenging in the case of coronaviruses as they are known to recombine in nature (Tao et al., 2017), and an attenuated vaccine strain could, in theory, recombine with wild coronaviruses to recreate a pathogenic strain. So far, there are only three attenuated SARS-CoV-2 vaccines generated by codon deoptimization under preclinical development, by Mehmet Ali Aydinlar University in Turkey, Codagenix and Serum Institute of India, and Indian Immunologicals Ltd and Griffith University.

Recombinant viral-vectored vaccines

Recombinant viral-vectored vaccines are built on either a replication-deficient viral backbone or an attenuated replication-competent viral backbone that is bioengineered to express antigens derived from the target pathogen. Although only a couple of viral-vectored vaccines have been approved for human use for the control of infections such as Ebola, this platform has been widely investigated and has a well-established track record for infectious diseases and cancer, given its genetic malleability, safety and ability to induce strong T cell responses without the need for an adjuvant (Humphreys et al., 2018). Some viral vectors, such as Ad5 and ChAd, usually need to be administered only once for protection and have natural tropism for the respiratory mucosa, which means they are amenable to respiratory mucosal vaccination (Afkhami et al., 2016). The technology already exists for their large-scale clinical grade production and storage.

Thus, recombinant viral vectors are the second most common platform for COVID-19 vaccine development, with 4 candidates currently in clinical trials, 38 under preclinical development and 3 (ChAdOx1 nCoV-19, Ad26-S and VSV-S) selected for US Operation Warp Speed. The non-replicating viral platforms are mostly based on Ad5 or MVA, and most of these vaccine

candidates express the S protein or RBD of SARS-CoV-2. Replication-competent viral vectors are mainly based on the vaccine strains of other human pathogens (such as measles or influenza viruses) or veterinary pathogens (such as vesicular stomatitis virus (VSV)). However, it will be important to consider whether humans have pre-existing immunity against the viral backbone. Pre-existing antibodies can impair the ability of such vaccines to engage the immune system. Use of viral backbones such as ChAd, for which humans have little to no pre-existing immunity, can help to circumvent this issue (Afkhami et al., 2016).

Ad5-nCOV, which is being developed by the Chinese vaccine company CanSino Biologics, is designed to induce neutralizing antibodies to SARS-CoV-2 S protein following intramuscular injection. Without published preclinical data, it entered phase I/II clinical trials with three doses of vaccine tested (Zhu et al., 2020). Of note, these doses are 10–30 times higher than those used in previous trials of intramuscular vaccines (Smaill et al., 2013). Whereas the highest dose generated unacceptable toxicity and was dropped from the phase II study, the smaller doses induced S protein-specific neutralizing antibodies in only 50% of the vaccine recipients (Zhu et al., 2020). The phase II study largely reaffirms the phase I observations that, although the vaccine induces antibody and T cell responses, its potency is reduced by pre-existing immunity to Ad5, particularly in elderly participants. Depending on geographical region, 35–95% of humans have significant circulating levels of neutralizing antibodies to Ad5. This is consistent with the rapidly declining antibody titres observed in a phase II Ad5-Ebola vaccine study (Zhu et al., 2017). The vaccine is entering further advanced trials in China and Canada, but the efficacy of this strategy is now in question. Another human adenovirus-based COVID-19 vaccine, known as Ad26-S, is being developed by Johnson & Johnson, although there is still 40% seroprevalence for Ad26 in humans. As Ad26 is inherently less immunogenic than Ad5, effective immunity requires repeated homologous or

heterologous vaccination, as has been shown in Ad26-HIV and Ad26-Ebola vaccine studies in human (Baden 2013). Nevertheless, a single parenteral administration of an Ad26-vectored COVID-19 vaccine (Ad26.COV2.S) offered robust protection in a non-human primate model of SARS-CoV-2 (Mercado 2020).

ChAdOx1 nCoV-19 (also known as AZD-1222), which is being developed by Oxford University, UK, and AstraZeneca, is the most clinically advanced COVID-19 vaccine. Humans have low sero-prevalence for ChAd, hence its strong immunogenicity and utility for heterologous prime–boost COVID-19 vaccination (Afkhami *et al.*, 2016). The development of ChAdOx1 nCoV-19 is based on promising human studies with ChAdOx1-MERS vaccine and ChAdOx1-TB vaccine. However, although intramuscular delivery of ChAdOx1 nCoV-19 reduced SARS-CoV-2 viral load in the lungs and prevented pneumonia in rhesus macaques, it did not reduce viral loads in the upper respiratory tract. A recently reported phase I/II study shows its safety and the induction of potent neutralizing antibody and T cell responses following a single parenteral injection, which are boosted further by a second homologous vaccination (Pedro *et al.*, 2020). It remains unclear from this trial to what extent both CD4⁺ and CD8⁺ T cell subsets were activated.

VSV-S is a replication-competent COVID-19 vaccine under development by Merck and other groups. Merck's vaccine is built upon the licensure of its highly efficacious VSV-Ebola vaccine, which induces neutralizing antibodies and cellular immunity against Ebola virus surface glycoprotein. VSV is a veterinary virus to which humans have no pre-existing immunity. However, the cloning capacity of the VSV vector is limited to 4 kb, and its suitability for respiratory mucosal vaccination is unclear. A single parenteral vaccination with a VSV vector expressing S protein provides protection against SARS-CoV-2 in both mouse and hamster models. Among other viral-vectored candidates is non-replicating MVA. MVA has widely been explored as a vaccine carrier and has a cloning capacity of up to 30 kb. However, as it is not robustly

immunogenic, MVA is often used as a booster vaccine or repeated injection is required to be effective, as was the case in clinical testing of an MVA-MERS-S vaccine (Pedro *et al.*, 2020).

Inactivated viral vaccines

Physically or chemically inactivated viruses have been used successfully in human vaccines against polio, hepatitis A and influenza. Inactivated viruses can be rapidly generated and scaled up in a pandemic situation using well-established infrastructure and method. Inactivated viral vaccines have few safety concerns, unlike their live attenuated counterparts, and they express a wide range of native viral antigens, including surface antigens with retained epitope conformations that can induce conformation-dependent antibody responses (Watanabe *et al.*, 2020).

Currently, there are five early clinical trials to assess inactivated SARS-CoV-2 vaccines, with an additional nine candidates in preclinical development⁵. PiCoVacc, an inactivated SARS-CoV-2 and alum-adjuvanted vaccine developed by Sinovac Biotech Ltd in China, is the most advanced candidate with published preclinical results. It protects rhesus macaques against SARS-CoV-2, with reduced viral titres and immunopathology associated with antibodies to S protein and nucleocapsid. BBIBP-CorV, another inactivated virus candidate, which is being developed by Chinese state-owned Sinopharm, was tested in a range of animal models, with demonstrated efficacy in non-human primates. Although these findings provide optimism, the observations were made in rather short-term studies and should be interpreted with caution.

Inactivated viral vaccines often require an adjuvant and repeated administration to be effective. The use of alum as an adjuvant makes them unsuitable for respiratory mucosal delivery (Zeng *et al.*, 2016). Although the protection mediated by intramuscular immunization with PiCoVacc or BBIBP-CorV indicates some level of mucosal immunity, probably through the transport of systemic antibodies to the lungs, the durability of such immunity remains unclear as

SARS-CoV-2 challenge was performed 1–4 weeks after vaccination. Furthermore, similarly to protein subunit vaccines, inactivated viral vaccines are poor inducers of cytotoxic CD8+ T cells, which are likely to be required for an effective COVID-19 vaccine. Studies with inactivated SARS-CoV and respiratory syncytial virus vaccines have reported vaccine-related enhancement of disease, likely involving a TH2 cell response and lung eosinophilia, which may be worsened in aged hosts. Although PiCoVacc or BBIBP-CorV did not worsen lung disease within 7 days after infection, alum is known to drive TH2 cell-mediated immune responses, which warrants further safety investigations. The use of TH1 cell-skewing modified alum or other adjuvants such as CpG may avert such safety concerns (Del Giudice et al., 2018).

Protein subunit vaccines

Currently, there are seven COVID-19 subunit vaccines in clinical trials, with 50 other candidates under preclinical development, making this the most common platform⁵. Subunit vaccines primarily induce CD4+ TH cell and antibody responses. Therefore, most of these vaccines contain full-length SARS-CoV-2 S protein or portions of it with the goal of inducing neutralizing antibodies, similarly to the majority of SARS and MERS vaccines, which had differing levels of efficacy (Zhou et al., 2018).

Subunit vaccines can be designed to focus the immune response towards neutralizing epitopes, thereby averting the production of non-neutralizing antibodies that may promote ADE of disease (Oscherwitz et al., 2016). However, unlike nucleic acid-based or viral-vectored vaccines, recombinant S proteins in subunit vaccines could have an improper epitope conformation unless they are produced in mammalian cells. Proteins or peptides alone are poorly immunogenic and generally require not only an adjuvant but also repeated administration, and they are poor activators of CD8+ T cell responses. Furthermore, this platform is generally unsuitable for respiratory mucosal vaccination. As is the case for inactivated viral vaccines, use

of unmodified alum as an adjuvant skews the immune response towards TH2 cell-like responses⁵⁶, which is undesirable for host defense against SARS-CoV-2 and may have a role in ADE of disease (Del Giudice et al., 2018). In this regard, subunit COVID-19 vaccines being developed by GlaxoSmithKline and Novavax use AS03 and Matrix-M adjuvants, respectively.

Virus-like particles (VLP)

VLPs are spontaneously forming particles composed of several structural viral proteins that are co-expressed or admixed. Several commercial vaccines, such as hepatitis B and human papillomavirus vaccines are based on VLPs (Donaldson et al., 2018). In the case of enveloped coronaviruses, VLPs form when the viral proteins S, M and E, with or without N, are co-expressed in eukaryotic producer cells. This results in active budding from the producer cells of VLPs that are structurally identical to the infectious virus but lack the viral genome and thus are non-infectious. The presence of S protein on the surface of VLPs enables them to bind and enter ACE2+ cells in the same manner as the parent virus. Unlike subunit vaccines, the array of S protein on the VLP surface crosslinks the B cell receptor and directly activates B cells, but, like subunit and inactivated viral vaccines, VLPs also typically require an adjuvant and repeated administration (Donaldson et al., 2018). Notwithstanding this, the VLP technology is well established, the biology and safety of coronavirus VLPs are understood and their large-scale production to Good Manufacturing Practice standards is relatively straightforward.

Currently, there is only 1 VLP-based COVID-19 vaccine in clinical trials, with 12 more under preclinical development. These are produced either in vivo from a viral vector, such as MVA, that expresses the VLP components (a platform being developed by GeoVax) or more often in vitro from producer cells. Notably, Medicago, a Canadian company, produces its SARS-CoV-2 VLPs from genetically engineered plants. Its unpublished results seem to suggest efficacy in inducing neutralizing antibodies in mice.

Nucleic acid-based vaccines

Recombinant plasmid DNA has been explored as a vaccine platform for decades, whereas mRNA has emerged more recently as a promising platform^{142,143}. Currently, there are 6 mRNA-based COVID-19 vaccines and 4 DNA-based COVID-19 vaccines in clinical trials, with 27 such vaccines (16 mRNA-based and 11 DNA-based vaccines) under preclinical development⁵.

The antigen-encoding mRNA complexed with a carrier such as lipid nanoparticles can be efficiently delivered *in vivo* into the cytoplasm of host cells for protein translation and post-translational modifications (Pardi *et al.*, 2018), which is an advantage over recombinant protein subunit vaccines. mRNA vaccines are non-infectious and are synthesized by *in vitro* transcription, free of microbial molecules. These beneficial features differentiate mRNA vaccines from live attenuated viral vaccines, inactivated viral vaccines, subunit vaccines and recombinant viral-vectored vaccines in terms of safety, efficacy and issues of antivector immunity, enabling their rapid and inexpensive production and repeated vaccination (Pardi *et al.*, 2018). mRNA-1273, which is produced by Moderna, an American biotech company that has experience with mRNA-based MERS vaccines, encodes a prefusion-stabilized SARS-CoV-2 S protein encapsulated in lipid nanoparticles. It entered clinical testing even before the release of preclinical data. Recently published phase I clinical trial data indicate that low and medium doses of two repeated parenteral injections are generally safe and induce strong S protein-specific antibody responses and a primarily CD4+ T cell response in most trial participants. Pfizer and BioNTech are also assessing an mRNA–lipid nanoparticle vaccine encoding the S protein RBD (known as BNT162b1) in humans, who developed robust S protein-specific antibody and CD4+ and CD8+ T cell responses following two repeated parenteral injections. The Pfizer/BioNTech and Moderna vaccines have both been selected for US Operation Warp Speed (Sahin *et al* 2020).

Although no mRNA vaccine has yet been licensed for human use, their potential is supported by previous studies of influenza, rabies and Zika virus infections in animals (Jasdave *et al.*, 2016). For example, an mRNA vaccine for influenza virus induced long-term humoral immunity in young and aged mice, and an mRNA vaccine for Zika virus induced both antibodies and cytotoxic CD8+ T cells in mice. However, two clinical studies show disparities in the magnitude and longevity of immune responses induced by mRNA vaccines. Thus, although mRNA-based COVID-19 vaccines show promise from early clinical testing, questions remain about their protective efficacy in humans. It is also unclear whether mRNA vaccines are amenable to respiratory mucosal delivery.

Plasmid DNA vaccines share several characteristics with mRNA vaccines, including safety, ease of production and scalability. However, they are poorly immunogenic, requiring multiple doses and the addition of an adjuvant. Currently, there are four plasmid DNA-based COVID-19 vaccines in clinical testing, with 11 more under preclinical development. INO-4800, a plasmid DNA vaccine expressing SARS-CoV-2 S protein, is being developed by the US biotech company Inovio Pharmaceuticals. A preclinical study in mice and guinea pigs examined the immunogenicity of this vaccine but did not provide any data pertaining to protection against challenge. Two repeated injections of an S protein-expressing plasmid DNA vaccine resulted in robust protective immunity in rhesus macaques.

Limitations of COVID Vaccines:

There are currently no immune correlates of protection for SARS-CoV-2 or other human coronaviruses. Thus, it is unclear what titre of neutralizing antibodies is sufficient to confer protection against infection. Establishing such correlates will be essential to guide the development of effective COVID-19 vaccines. the inclusion of other structural (N) and/or non-structural proteins as vaccine antigens may help to create a more balanced response involving both

humoral and T cell-mediated immunity. These could be highly expressed proteins such as N protein or highly conserved functional proteins that have a crucial role in the viral life cycle. In broad terms, vaccines require two components: antigens from the target pathogen that are provided to or generated by the vaccine recipient; and an infection signal (such as a pathogen-associated molecular pattern or damage-associated molecular pattern) that alerts and activates the host immune system. Live attenuated vaccines can naturally provide both of these components, whereas non-viral vaccine platforms can provide the antigens but often require the artificial provision of signals to alert the immune system known as adjuvants. Typically, these non-viral vaccine platforms require multiple vaccinations to induce protective immunity, whereas live virus-based vaccines have the ability to provide 'one-shot' immunity. Similarly to non-viral platforms, killed virus vaccines sometimes require the inclusion of an adjuvant and repeated administration for full efficacy. There have been several cases of vaccinated people, even those who have received both doses, testing positive for the virus. Such cases are referred to as "breakthrough" infections, indicating that the virus has been able to break through the defenses created by the vaccine. According to Center for disease control (CDC) data that was released this week, over 87 million Americans were fully vaccinated and 7,157 cases of Covid-19 were reported among them. The Indian Council of Medical Research (ICMR) released data that showed breakthrough infections were extremely rare in India's vaccinated population, with an incidence rate of less than 0.05%. Until then, 11.6 crore doses of the Covishield vaccine had been administered. Among 10.03 crore people who had taken only the first dose, 17,145 had got infected. That translates into a 0.02% prevalence. Among the 1.57 crore people who received the second dose as well, 5,014, or about 0.03%, had got infected later. Again, about 1.1 crore doses of Covaxin have been administered until now. Of the 93.56 lakh who took only the first dose, so far 4,208 have got the infection. That is about 0.04% of the total. Among the 17.37 lakh who have

taken the second shot, only 695 had been infected, again 0.04%. The researchers reported that, among the people who developed symptoms more than 21 days post-vaccination, 113 (of 400) died with covid-19 (28%). Of these, 82 were in the "frail elderly" group. The report said, "Mortality appears to remain high for people in high risk vaccination tiers who are admitted to hospital with SARS-CoV-2 infection despite vaccination 21 day or more previously (Elisabeth Mahase., 2021)

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