REVIEW

Pandemic COVID-19 caused by SARS-CoV-2: genetic structure, vaccination, and therapeutic approaches

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Abstract
We give a summary of SARS-genetic CoV-2's structure and evolution, as well as current attempts to develop efficient vaccine and treatment methods for SARS-CoV-2 infection, in this article. Most therapeutic strategies are based on repurposing of existing therapeutic agents used against various virus infections and focused mainly on inhibition of the virus replication cycle, enhancement of innate immunity, and alleviation of CRS caused by COVID-19. Currently, more than 100 clinical trials on COVID-19 aim to provide robust evidence on the efficacy of the currently available anti-SARS-CoV-2 antiviral substances, such as the nucleotide analogue remdesivir, the antimalarial drug chloroquine, and drugs directed against docking of SARS-CoV-2 to the membrane-associated angiotensin-converting enzyme 2 (ACE2) such as transmembrane protease serine 2 (TMPRSS2). The current vaccination campaign is ongoing worldwide using different types of vaccines such as Pfizer-BioNTech and Moderna, Johnson & Johnson, Oxford-AstraZeneca, Novavax, and others with efficacy ranging from 72–95%. In March 2021 Germany limited the use of the Oxford-AstraZeneca COVID-19 vaccine to people 60 years of age and older due to concerns that it may be causing blood clots. Further study and more data are needed to confirm the safety of different available vaccines.

Keywords COVID-19 genomics · Vaccine · Antiviral agents · Therapeutic approach

Abbreviations

SARS-CoV2	Severe Acute Respiratory Syndrome Coronavirus 2
COVID-19	Corona Virus Disease 2019
ARDS	Acute respiratory disease syndrome
CDC	Center for Disease Control
HLA	I human leukocyte antigen
RTC	Replication-transcription complex
DMVs	Double-membrane vesicles
sgRNAs	Subgenomic RNAs
SARS-CoV	Severe Acute Respiratory Syndrome Coronavirus
MERS-CoV	Middle East Respiratory Syndrome
HKU-1	Hongkong University-1
ORF	Open reading frame
ACE2	Angiotensin 2 Converting Enzyme
CEPI	Epidemic Preparedness Innovations
aAPCs	Artificial antigen-presenting cells
CRS	Cytokine release storm
HCV	Hepatitis virus C
RSV	Respiratory syncytial virus
HIV	The human immunodeficiency viruses
FDA	Food and Drug Administration

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Introduction

At the end of 2019, an alarming infectious disease caused by a newly discovered coronavirus was first recorded in the Chinese city of Wuhan (Hubei province, [1]). The rapid, disturbing world- wide transmission of this virus, named COVID-19, resulted in declaration of a global health emergency by the World Health Organization on 30 January 2020 [2]. As of April 1, 2021, there have been 129 M global confirmed cases, 2.82 M recovered cases of COVID-19, and 2.82 M deaths reported to WHO. Mutti et al. [3] reported that ~ 18% of COVID-19 patients develop interstitial pneumonia, and ~ 5% of them develop acute respiratory disease syndrome (ARDS), which is mostly fatal [3]. The risk of ARDS rises with age, and almost all deaths are seen in patients with pre-existing chronic conditions [19, 20].

Increasing evidence has shown sustained human-to-human transmission, resulting in many cases exported across the globe by travelers (CDC, 2020). A role for specific class I human leukocyte antigen (HLA) alleles in shaping the anti-viral immune response to COVID-19 has been suggested, and permissive roles of HLA-C*01 and B*44 towards SARS-CoV-2 infection have also been postulated [4]. This information may help to identify sub-populations at risk, facilitating the prioritization of vaccination programs.

In this review we provide a brief introduction of the epidemiology of SARS-CoV-2 and describe its theoretical potential evolution, based on what is known about preexisting similar coronaviruses. We provide an update on the potential origin and evolution of the mutated genetic structure of SARS-CoV-2, and of the current efforts to provide effective vaccination and therapeutic strategies.

Genome structure of SARS-CoV-2 compared to other coronaviruses

The production of an efficient vaccine/treatment necessitates a thorough understanding of the genetic, genomic and proteomic architecture of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV2). According to the phylogenetic tree of coronaviruses (CoVs), CoVs belong to the order Nidovirales, which includes the family Coronaviridae and the subfamily Coronavirinae. Four genera are included in the subfamily Coronavirinae: Alphacoronavirus, Betacoronavirus, Gammacoronavirus, and Deltacoronavirus [5].

The CoVs genome structure is ~ 30 kb single-stranded positive-sense RNA (+ ssRNA) with 3'-poly-A tail, and 5'-cap structure. The CoV's genome is translated into two long polypeptides (Pp1a and pp1b) that are processed into 16 nonstructural proteins (nsps) forming the replication-transcription complex (RTC) of double-membrane vesicles (DMVs, [6]). Adopting a discontinuous transcription mechanism, the RTC synthesizes a nested set of subgenomic RNAs (sgRNAs) that possess common 5'-leader and 3'-terminal sequences [7]. Five ORFs constitute the genome of CoV: The first ORF (ORF1a/b) encodes 16 nonspecific proteins (nsp1-16). Others encode at least four main structural elements: spike (S), membrane (M), envelope (E), and nucleocapsid (N) proteins [7, 8]. The S proteins form the spikes on the viral surface that mediate attachment of virions to host receptors [9–16].

The structural proteins include the structural Spike (S), Envelope (E), Membrane (M), Nucleocapsid (N), Hemagglutinin esterase (HE) and Helicase (H) proteins. The nonstructural proteins (nsp) include Proteases, papain-like proteases (PLP or PLpro) and 3C-like protease (3CLpro), and Replicase proteins [17]. The spike (S) protein protrudes on the viral surface, and play a crucial role in the fusion of the viral particles to specific receptors on host cells, while the envelope (E) protein is known to have viroporin activity which is essential for completion of the infection cycle. The nucleocapsid (N) protein merge with the viral single (+) stranded RNA genome forming a nucleoprotein complex which is maintained within the capsid comprised in part of the matrix (M) protein. The protease proteins have been a target of anti-viral action by lopinavir/ritonavir, with and without arbidol, an anti-envelope viral indole derivative [18].

Evolution and novel genetic structure of severe acute respiratory syndrome coronavirus 2 (SARS-CoV2)

By the end of January 2020, the genomic sequence of SARS-CoV-2 was determined and the information released [19]. To understand the origin of this novel coronavirus and how it is related to existing coronavirus members, the genomic sequences of SARS-CoV-2 were compared to the severe acute respiratory syndrome (SARS) caused by a SARS-associated coronavirus that was first identified at the end of February 2003, and Middle East respiratory syndrome or (MERS-CoV) that was first identified in Saudi Arabia in 2012 [20]. Interestingly, the sequence of SARS-CoV-2 shared a higher homology with SARS-CoV than that of MERS-CoV. Moreover, poor sequence homology was noted mainly in the ORF1ab and spike (S-protein) gene. Bat-CoV and SARS-CoV-2 showed 92% sequence similarity. MERS-CoV and SARS-CoV-2 nucleocapsid analysis indicated only 65% identity. Molecular characterization of nucleocapsids from various coronaviruses revealed that SARS-CoV 2 is more related to SARS-CoV 1 and bat-CoV. SARS-CoV 2 exhibited less resemblance with MERS-CoV. SARS-CoV 2

showed less similarity to MERS-CoV. Thus, either SARS-CoV-1 or bat-CoV may be the source of SARS-CoV-2 evolution [21].

MERS-CoV was found to share genetic homology with many bat CoV species worldwide, which included the Egyptian tomb bats, African bats [22, 23], Italian bats [24], and Chinese bats [25]. Similar to SARS-CoV, MERS-CoV also has two domains S1 and S2, of which S1 is the variant peptide that binds to the receptor dipeptidyl peptidase 4 (DPP4). S1 domain is located in the 240 residue of the C-terminal receptor binding domain and are composed of a core and external subdomain unit enabling their attachment, fusion, and entry into the host cells [26].

Recently evolved SARS-CoV-2 (COVID-19), the seventh known coronavirus has shown similar properties of SARS-CoV with the presence of a highly variable S1 sub-unit of spike protein. It was also found that about five of the six residues of S1 domain differ in SARS-CoV-2 compared to the S1 domain of SARS-CoV virus [27]. Further, the presence of polybasic cleavage site namely the three O-linked glycan in the intersection of the S1 and S2 sub-domains also appear to play a vital role in the infective properties of the virus [28]. Thus, it is inferred that the variation in the S1 and S2 domains of the spike protein act as the major site of mutation, insertion, and deletion of genes during every transformation of the SARS-CoV-2 virus. This enables the evolution of the new infective virus forms that are capable of efficiently binding to the receptor binding domain, further enabling the effective invasion of viral particles into the human cells.

Whether or not the transmission pathway from bats to humans may include other intermediate hosts is still a matter of speculation. Based on genomic profiling of the two mostly related human- infecting SARS-CoV, it is now evident that the genetic distance of SARS-CoV-2 is much closer to the human-infecting SARS-CoV than is the MERS-CoV [29]. Although the origin of the virus remains unresolved, Wacharapluesadee et al. extended the geographic distribution of genetically diverse SARS-CoV from Japan and China to Thailand over a 4800-km range. Cross-border surveillance is urgently needed to find the immediate progenitor virus of SARS-CoV-2 [30].

In a recent report, WHO-convened global study of origins of SARS-CoV-2. The study aimed at identifying the zoonotic source of SARS-CoV-2, the route of introduction to the human population, and potential intermediate hosts. This is crucial to reduce further risks of the emergence of new virus zoonotic disease. The study concluded that at this stage, it is not possible to determine precisely how humans in China were initially infected with SARS-CoV-2. However, all available evidence suggests that SARS-CoV-2 has a natural animal origin and is not a manipulated or constructed virus. SARS-CoV virus most probably has its ecological reservoir in bats [31].

Coronaviruses are known to utilize S-protein to facilitate entry into the host cells by binding to host receptors. The two functional units of the S-proteins (S1 and S2) play crucial roles in host receptor binding. Individually, the C-terminal RBD is directly attached to surface receptors [32]. The S-protein has been reported to have the most variable sequence of amino acid residues [33]. Despite the observation that the SARS-CoV-2 S-protein has a low overall homology to SARS-CoV, the RBD domain of SARS-CoV-2 does show several sequences with close homology to that of SARS-CoV_Tor2 and HP03-GZ01. The receptor complex of SARS-CoV S-protein has five amino acids residues that are crucial for human-to-human and cross-species transmission [34]. In the SARS-CoV-2 S-protein, Tyr491 is the only conserved residue; the other 4 residues are not preserved. Similar to SARS-CoV, SARS-CoV-2 also uses the angiotensin-converting enzyme 2 (ACE2) for cell entry [35].

To prove the ability, and the nature, of the interaction of SARS-CoV-2 with human ACE2 molecules, Xu et al. [36] have studied the structural model of the SARS-CoV-2 S-protein using a computer-guided homology model based on the crystal structure of SARS coronavirus S-protein (PDB accession: 6ACD) as a template [37].

Repurposing existing antiviral therapy for COVID-19

Until now, no specific antiviral therapy has been available to treat COVID-19 respiratory damage. Nevertheless, efforts are ongoing to test several antiviral drugs both at the preclinical and clinical levels. Based on their mode of action, anti-COVID-19 therapies might target either enzyme involved in viral replication; or interfere with the viral entry into the host cells. Others might target the host immune system to enhance host immunity; or inhibit the cytokine release storm (CRS) associated with the massive damage of lung tissues (Fig. 1).

Based on previous therapeutic experience with SARS and MERS, several options might be available: these include small-molecule antiviral drugs, monoclonal antibodies, interferon therapies, oligonucleotide-based therapies, peptides, and vaccines [38].

Based on the genomic code of SARS-CoV-2, a set of structural and non-structural proteins have been identified as potential therapeutic targets. Replicating enzymes (3-chymotrypsin-like protease, papain-like protease, helicase, and RNA-dependent RNA polymerase) that are essential in the viral life cycles as well as S protein, which is known to play a decisive role in SARS-CoV-2 entry into the host cells via ACE2 receptor [39], may be options. Drugs directed against

Fig. 1 Various therapeutic targets for SARS-Co-v-2
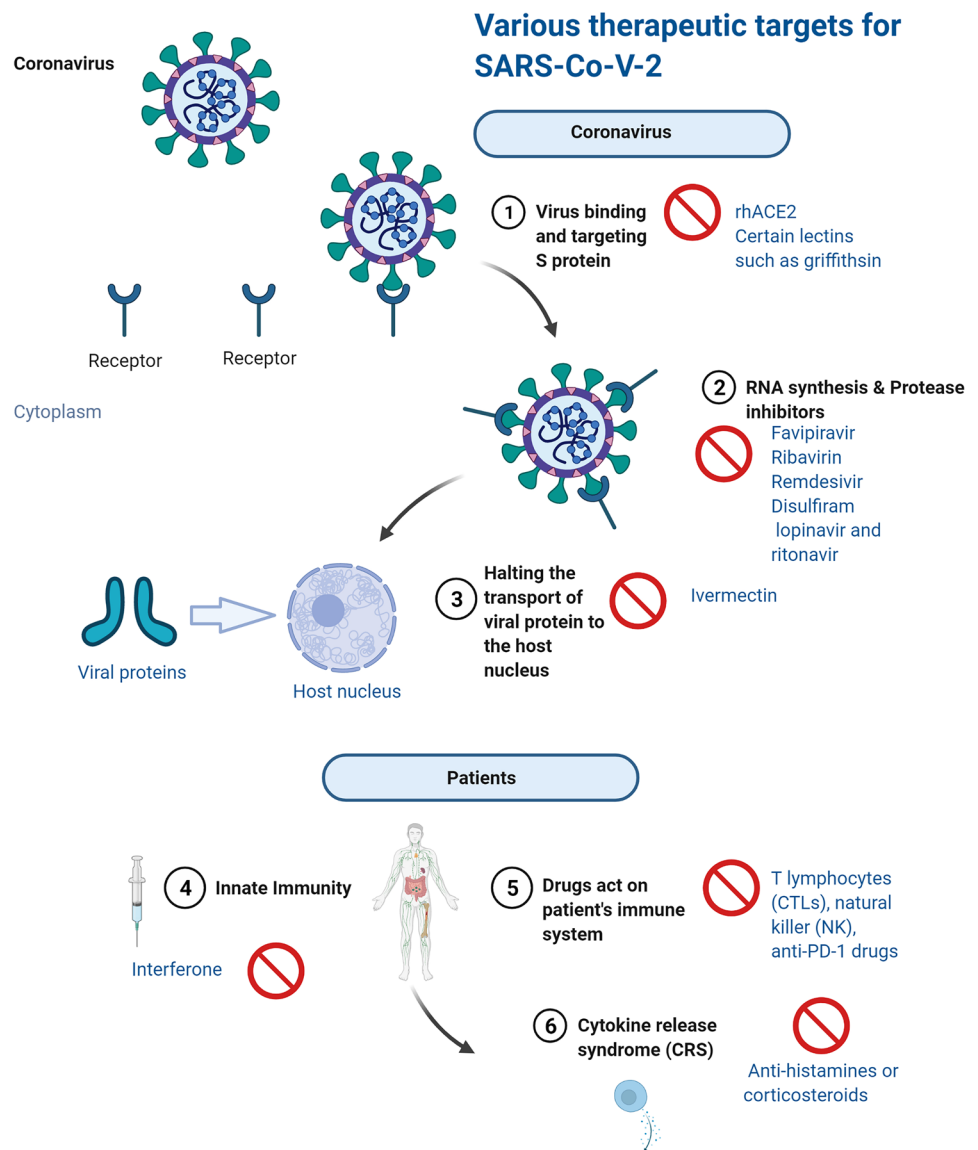
This figure shows the various therapeutic targets of SARS-CoV-2 and drugs used against them.

docking of SARS-CoV-2 to the membrane-associated angiotensin converting enzyme 2 (ACE2) such as transmembrane protease serine 2 (TMPRSS2) are also under current evaluation [40].

When comparing them to SARS and MERS enzymes, the catalytic sites of the SARS-CoV-2 replication enzymes and the key drug-binding pockets in viral enzymes seem to be highly conserved. Therefore, repurposing existing MERS and SARS antiviral agents such as nucleoside analogues (favipiravir and ribavirin) and/or experimental nucleoside analogues (remdesivir and galidesivir) may be a promising therapeutic approach (Fig. 1). The nucleoside analogues act against a wide range of RNA viruses (including coronaviruses) principally by targeting the RNA-dependent polymerase, thus inhibiting viral RNA synthesis [41].

The antiviral inhibitory effect of favipiravir (T-705) has been confirmed against several RNA viruses such as influenza, Ebola, yellow fever, chikungunya, norovirus and enterovirus. Favipiravir has been approved to treat influenza [41]. Using Vero cells, favipiravir has been demonstrated to be effective against SARS-CoV-2 (EC50 = 61.88 µM in Vero E6 cells). It effectively inhibits the RNA-dependent polymerase of SARS-CoV-2 by structurally resembling endogenous guanine [34, 42]. Currently, the efficacy of favipiravir plus interferon-α (ChiCTR2000029600) and favipiravir plus baloxavir marboxil (ChiCTR2000029544) are being evaluated in COVID-19 patients.

Ribavirin is approved for hepatitis virus C (HCV) and respiratory syncytial virus (RSV). The efficacy of ribavirin against SARS and MERS has also been evaluated, but it has

been reported to induce severe anaemia when administered in a high dose [39]. Whether or not ribavirin can confer efficient potency against SARS-CoV-2is has yet to be clarified.

Remdesivir (GS-5734) is a prodrug whose structure resembles adenosine. As an adenine derivative, it was initially developed to treat Ebola [43]. Previous studies have proven that remdesivir induces productive antiviral activity against SARS-CoV and MERS-CoV, in vitro and in vivo [44, 45]. A recent study showed that remdesivir inhibited SARS-CoV-2 (EC50 = 0.77 μM in Vero E6 cells, [42, 46]). A COVID-19 patient has recovered after receiving intravenous remdesivir [47].

Galidesivir (BCX4430) as an adenosine analogue has been evaluated against HCV and yellow fever, and has been proven to provide antiviral effects against SARS and MERS [39].

Protease inhibitors are used against several virus infections and disease conditions such as SARS, MERS, HIV, and alcohol dependence. The rationale behind the use of protease inhibitors (such as disulfiram, lopinavir and ritonavir) is their ability to inhibit papain-like protease. The protease proteins have been a target of anti-viral action by lopinavir/ritonavir, with and without a rbidol, an anti-envelope viral indole derivative [18]. Inhibition of MERS and SARS papain-like protease has been demonstrated in cell cultures, but clinical evidence still needs to be developed. Currently both lopinavir and ritonavir, previously tested as HIV protease inhibitors, are under clinical trial in COVID-19 patients. In a non-randomized open label trial, lopinavir and ritonavir administration were associated with improved clinical outcome in SARS patients, and have been hypothesized to induce such favorable outcomes via inhibition of 3-chymotrypsin-like protease [39]. Whether HIV protease inhibitors can improve the clinical outcome in COVID-19 patients is still in need of clarification.

Targeting the S protein of SARS-CoV-2 is another tactic to prevent viral entry into the host cells. Certain lectins (such as griffithsin, which is derived from red algae) have been reported as able to bind to oligosaccharides associated with the HIV glycoprotein 120 and SARS-CoV S glycoprotein [39]. In a phase I clinical trial griffithsin has been evaluated against HIV using different routes of administration as gels or in enemas. Whether the same approach could be implemented for SARS-CoV-2 is still in need of study.

The FDA-approved anti-parasitic ivermectin has previously shown antiviral effects against several RNA viruses such as HIV and dengue virus [48]. Ivermectin acts by dissociation of the IMPα/β1 heterodimer, thereby halting the transport of viral protein cargo to host cell nuclei. This results in inhibition not only of the viral replication cycle but also the endogenous antiviral defense mechanisms in the host cells. In a recent in vitro study, ivermectin was able to reduce the viral RNA load up to 5000-fold 48 h after

infection with SARS-CoV-2 [49]. The next logical step after establishing the safety profile and efficacy against SARS-CoV-2 would be the initiation of clinical testing to estimate the appropriate dose necessary to inhibit the SARS-CoV-2 viral replication cycle.

Inhibition of viral entry into host cells by blocking virus-cell membrane fusion seems to be another promising approach against SARS-CoV-2 infections. Molecules designed to occupy the ACE2 receptors in competition with SARS-CoV-2 might inhibit the ability of SARS-CoV-2 to enter the host cells. Soluble recombinant human Angiotensin-converting Enzyme 2 (rhACE2) blocks the interaction between the S protein of SARS-CoV-2 and ACE2, and inhibits SARS-CoV-2 replication in cellular and embryonic stem cell-derived organoids by a factor 1000–5000 times [50]. Administration of rhACE2 preserved the integrity of pulmonary vasculature and prevented acute respiratory distress syndrome (ARDS, [51]). Currently, the biological and physiological role of rhACE2 is being evaluated in a small pilot study in China (NCT04287686), and the safety and tolerability of APN01 (rhACE2 produced by Apeiron Biologics) are currently being assessed in a placebo-controlled, double blinded, dose-escalation clinical trial.

Glycosylation of ACE2 receptors has also been demonstrated to inhibit the replication of SARS-CoV [52].

The cytotoxic T lymphocytes (CTLs) and natural killer cells (NK) are known to have an essential role in fighting viral infection. In SARS-CoV-1 infection [53], and tumorigenesis [54] persistent immune activation is associated with lymphocyte exhaustion, showing significantly higher levels of exhaustion markers. For example programmed death-1 (PD-1), is higher compared to healthy controls [55]. The use of anti-PD-1 drugs to trigger exhausted T cells by blocking PD-1 in cases of viral infections has been suggested and the same concept might be applied to SARS-CoV-2 infection [56].

Recently, a solidarity clinical trial for COVID-19 treatments has been launched by the World Health Organization and partners to evaluate the effects of drugs used to treat COVID-19 on three important outcomes in COVID-19 patients: mortality, need for assisted ventilation and duration of hospital stay. The solidarity study concluded that all 4 treatments evaluated (remdesivir, hydroxychloroquine, lopinavir/ritonavir and interferon) had little or no effect on overall mortality, initiation of ventilation and duration of hospital stay in hospitalized patients (https://www.who.int/emergencies/diseases/novel-coronavirus-2019/global-research-on-novel-coronavirus-2019-ncov/solidarity-clinical-trial-for-covid-19-treatments). Other studies have shown the inefficacy of Hydroxychloroquine in COVID-19 patients [57].

Ongoing SARS-CoV-2 vaccination

Elucidation of the genomic sequence of SARS-CoV-2, first published on 11 January 2020, was the first crucial step toward designing genome-based specific vaccines and therapeutic antibodies. The next step is to test the safety and efficacy of such novel vaccines in clinical trials. Although this represents the classical pathway of vaccine development, given the current pandemic nature of SARS-CoV-2, this long-term solution, while needed, is a problem. Much more rapid intervention is urgently required to halt a lethal pandemic.

Based on data released by The Coalition for Epidemic Preparedness Innovations (CEPI, a collaborative effort between global health authorities and vaccine developers to support the development of vaccines against COVID-19) the vaccine R&D landscape now includes 115 vaccine candidates. These include approaches using nucleic acid (DNA and RNA), virus-like particles, peptides, viral vector recombinant protein (replicating and non-replicating), as well as live attenuated-virus and inactivated-virus (Fig. 2 and Table 1).

The DNA and mRNA vaccines have significant advantages due to the ease of antigen manipulation and potential for speedy production. Viral-vector-based vaccines provide a much stronger immune response, higher levels of protein expression, and therefore long stability. Adjuvant vaccines against COVID-19 are expected to enhance the immune response and decrease the vaccine dose. The crucial role played by the viral spike (S) protein in viral uptake via the ACE2 receptor has prompted the creation of vaccines targeting the S proteins. These are intended to trigger the production of neutralizing antibodies against it. That would prevent the SARS-CoV-2 uptake via the ACE2 receptor. Whether the different variants of the S protein used in different vaccines under trial are related to each other or the genomic epidemiology of the disease is not yet clear.

Several B and T cells epitopes that map identically to SARS-CoV-2 proteins have been identified [58]. Immune targeting of these epitopes may potentially offer protection [58]. The time needed for traditional vaccine development would normally average 10 years, but given the lethal nature of the pandemic, unprecedented efforts have currently been employed to produce an effective vaccine by late 2020 and early 2021. The situation has necessitated adoption of a novel paradigm relevant not only in the development phase but also in scaling up manufacturing capacity. With respect to the concerted global efforts for speedy production of COVID-19 vaccine, it is crucial to assess efficacy through development of animal models. Furthermore, availability of Biosafety-level 3 facilities is crucial for performing animal studies involving live-virus challenges [59].

Demonstration of the crucial role played by the S protein in initiating of infections, together with the data generated by sequencing of the SARS-CoV-2 genome led many of the vaccine developers to assess the efficacy of multiple nucleic acid-based vaccine candidates based on the genomic sequence of S protein. The safety and efficacy of a synthetic mRNA (Moderna's mRNA-1273, NCT04283461) and synthetic DNA (Inovio Pharmaceuticals, INO-4800) encoding the S protein of SARS-CoV-2 are currently being tested.

Other lipid-nanoparticle-encapsulated S protein-based mRNA vaccines are also expected to trigger an effective antiviral immune response upon intramuscular injection. The immunogenicity of genetic vaccines depends to a great extent on the route of administration and the amount of delivered plasmid. In a Phase I/II an ongoing clinical trial, the genetic sequence of the S protein from the nCoV-19 was introduced into a non-replicating adenovirus vector. The adenovirus tropism to both respiratory and gastrointestinal epithelium (the two sites for which SARS-CoV-2 has a predilection) and its ability to exploit the ACE-2 receptor as its main entry route into the host cells potentially makes the non-replicating adenovirus an appropriate vector for a DNA-based vaccine. However, the potential for eliciting an immune response against the vector genes (and to a lesser extent against the transgenes) is still in need of clarification (NCT04324606).

Mimicking previous trials with influenza and Ebola viruses, a stabilized subunit vaccine based on molecular clamp technology is under trial for SARS-CoV-2. This approach might allow recombinant viral proteins to remain stable in pre-fusion form, allowing investigators to test their ability to induce the production of neutralizing antibodies [60].

By linking nanoparticles to SARS-CoV-2 S protein antigenic epitopes, a nanoparticle-based vaccine able to trigger an antigen-specific lymphocyte proliferation (as well as cytokine production) is expected to enter phase I trial this summer by Novavax, Inc [61]. The S protein epitopes are stably expressed in a baculovirus system [62].

Cytotoxic T cells and genetically modified artificial antigen-presenting cells (aAPCs) that are specific for SARS-CoV-2 S antigenic epitopes could trigger the naïve T cells in the human body, leading to their differentiation and proliferation. Ongoing clinical trials are now assessing the safety and immunogenicity of aAPCs alone and in combination with antigen-specific cytotoxic T cells (NCT04299724, NCT04276896).

The strategies adopted in the development of vaccines are very different from each other and consequently, so are the types of vaccine that can protect against infection (Fig. 2). In particular, the researchers are working on different types of vaccines: RNA vaccine, DNA vaccine, protein vaccine, inactivated viral vaccine, viral vector vaccine, attenuated
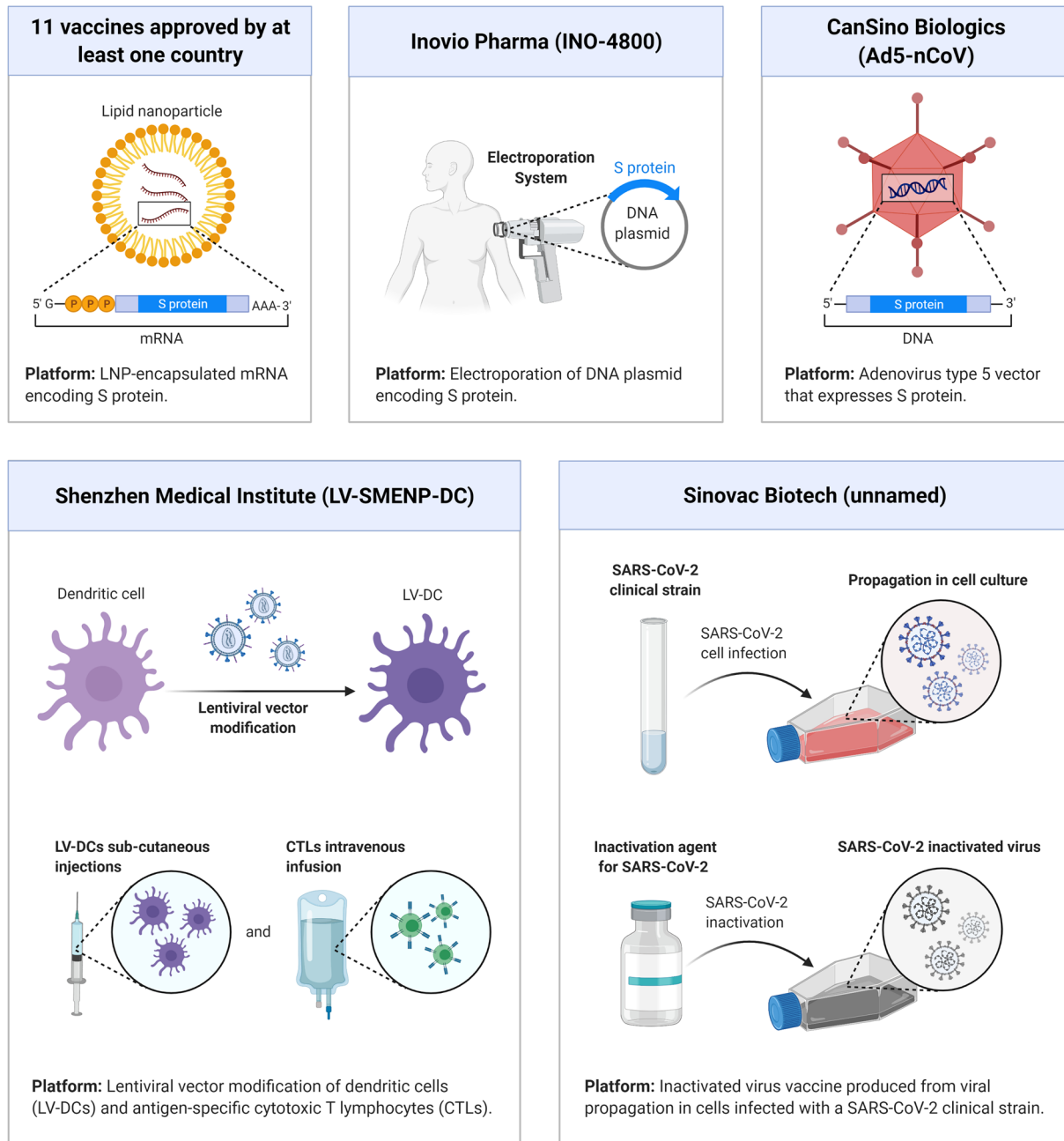
Fig. 2 Various types of vaccines candidate for COVID-19

live vaccine. However, the situation of vaccine development is rapidly and continuously evolving, as is the pandemic. Specifically, an overview of the clinical trial phases of the anti SARS—CoV2 vaccines is reported for the period of November/December 2020.

Worldwide, China currently presents two vaccines in phase 1/2 (SARS-CoV-2 Inactivated Vaccine, LV-SMENP DC) and two in phase 3 (Vero cell, Ad5-nCoV); the United States two in phase 3 (mRNA-1273 sponsor Moderna TX. Inc., AZD-1222 sponsor Astra Zeneca Iqvia Pty Ltd); India

Table 1 Various types of vaccines

No.	Name	Type	Approval	Trials/country
11 vaccines approved by at least one country				
1	FBRI EpiVacCorona	Protein subunit	1 country	2 trials in 1 country
2	Moderna mRNA-1273	RNA	38 countries	5 trials in 1 country
3	Pfizer/BioNTech BNT162b2	RNA	60 countries	9 trials in 9 countries
4	CanSinoAd5-nCoV	Non-Replicating Viral Vector	Approved in 3 countries	6 trials in 6 countries
5	GamaleyaSputnik V	Non-Replicating Viral Vector	Approved in 26 countries	15 trials in 6 countries
6	Oxford/AstraZenecaAZD1222	Non-Replicating Viral Vector	Approved in 48 countries	18 trials in 13 countries
7	Serum Institute of IndiaCovishield	Non-Replicating Viral Vector	Approved in 11 countries	2 trials in 1 country
8	Bharat BiotechCovaxin	Inactivated	Approved in 1 country	5 trials in 1 country
9	Sinopharm (Beijing)BBIBP-CorV	Inactivated	Approved in 14 countries	6 trials in 7 countries
10	Sinopharm (Wuhan)	Inactivated	Approved in 2 countries	6 trials in 7 countries
11	SinovacCoronaVac	Inactivated	Approved in 7 countries	11 trials in 5 countries
20 vaccines in phase 3 clinical trials				
12	NovavaxNVX-CoV2373	Protein subunit		**5 trials** in 6 countries
13	Anhui Zhifei LongcomRBD-Dimer	Protein subunit		**5 trials** in 1 country
14	MedicagoPlant-based VLP	VLP		**3 trials** in 2 countries
15	InovioINO-4800	DNA		**6 trials** in 3 countries
16	AnGesAG0301-COVID19	DNA		**4 trials** in 1 country
17	ModernamRNA-1273	RNA	Approved in 38 countries	5 trials in 1 country
18	CurevacCVnCoV	RNA		**5 trials** in 4 countries
19	Pfizer/BioNTechBNT162b1	RNA		**5 trials** in 3 countries
20	Pfizer/BioNTechBNT162b2	RNA	Approved in 60 countries	9 trials in 9 countries
21	GamaleyaSputnik V	Non-Replicating Viral Vector	Approved in 26 countries	15 trials in 6 countries
22	Janssen (Johnson & Johnson)Ad26.COV2.S	Non-Replicating Viral Vector		**6 trials** in 17 countries
23	CanSinoAd5-nCoV	Non-Replicating Viral Vector	Approved in 3 countries	6 trials in 6 countries
24	Oxford/AstraZenecaAZD1222	Non-Replicating Viral Vector	Approved in 48 countries	18 trials in 13 countries
25	Serum Institute of IndiaCovishield	Non-Replicating Viral Vector	Approved in 11 countries	2 trials in 1 country
26	Chinese Academy of Medical SciencesInactivated	Inactivated		**5 trials** in 3 countries
27	Kazakhstan RIBSPQazCovid-in	Inactivated		**3 trials** in 1 country
28	Bharat BiotechCovaxin	Inactivated	Approved in 1 country	5 trials in 1 country
29	Sinopharm (Beijing)BBIBP-CorV	Inactivated	Approved in 14 countries	6 trials in 7 countries
30	Sinopharm (Wuhan)Inactivated		Approved in 2 countries	6 trials in 7 countries
31	SinovacCoronaVac	Inactivated	Approved in 7 countries	11 trials in 5 countries

By 19 February 2021, 11 COVID-19 vaccines approved by at least one country, 20 vaccines in phase 3 clinical trials, 39 vaccines in phase 2 clinical trials, and 27 vaccines in phase 1 clinical trials

with two phase 1/2 vaccines (BBV-152, Novel Corona Virus. 2019- nCov Vaccine); Brazil one in phase 3 (PROFISCOV); Korea a phase 1/2 vaccine (INO.4800); Turkey one in phase 3 (CoronaVac); Russia one in phase 3 (Gam-COVID Vac). In Europe: United Kingdom one in phase 1/2 (LNP-nCoVs-aRNA sponsor Imperial College (London) and one in phase 2/3 (ChAdOx1 nCoV-19 sponsor University Oxford); Belgium and Germany one in phase 1/2 (CVnCoV Vaccine sponsor CureVac AG); Germany one in phase 3 (BNT 162b2 sponsor BioNTech SE + Pfizer). In Italy, AIFA with a statement authorized on 31/07/2020, the testing of phase I on the anti-COVID-19 vaccine produced by Italian Biotechnological company ReiThera. This is a phase I study which aims to evaluate the safety and immunogenicity of the GRAd-COV2 vaccine. The vaccine development project is supported by the Ministry of Research (MUR) with the National Research Council (CNR) of Italy and by the Lazio Region. The trial will be conducted at the National Institute for Infectious Diseases "L. Spallanzani" in Rome and at the Clinical Research Center in Verona (Table 1). The results of the phase 2/3 clinical trial have been published in a recent publication in "The Lancet" magazine. This referred to the ChAd vaccine 0×1 nCoV-19 from the Biopharmaceutical company Astra Zeneca on a study carried out on 4 cohorts, and showed significant efficacy of 70.4% after 2 doses and a protection level of 64.1% after at least one standard dose,

against symptomatic disease, without safety problems. Furthermore, according to the authors who followed the evolution of vaccines administration at various age groups of the population, they showed a greater tolerability for the vaccine in older adults than in younger adults. It also has similar immunogenicity in the three different age groups after the second dose. However, further studies are deemed necessary to evaluate the efficacy of the vaccine in individuals with comorbidities. On November 18, 2020, Pfizer published the preliminary results of the study in phase 3 (US National Library of Medicine ClinicalTrials.gov) announcing the conclusion that the trial has shown a 95% efficacy of the vaccine against COVID-19, starting on day 28 after administration of the first dose. When participants were age-stratified, the efficacy observed in adults over the age of 65 years was greater than 94%. Considering that the safety data required by the US Food and Drug Administration (FDA) for the authorization for emergency use (EUA) has been reached, the data were presented not only to these Authorities but also to other Agencies around the world, including AIFA in Italy and EMA in Europe. Regarding the Russian Sputnik V vaccine, preliminary data published on the website sputnikvaccine.com show an efficacy of over 95% at 42 days from the first dose. The preliminary results of the Phase 3 study by the Moderna company were also published, which reports that the 0.1773 mRNA vaccine is 100% effective against severe COVID and was found to be safe and well tolerated with an efficacy rate of 94.5%. (Fig. 2 and Table 1).

Vaccines Authorized by FDA (Food and Drug Administration), EMA (European Medicines Agency) and AIFA (Italian Medicines Agency), from December 2020 to January 2021

On 10 December 2020, the article "Safety and Efficacy of the BNT162b2 mRNA COVID-19" was published in The New England Journal of Medicine with the results of a Phase III study [54]. On 11 December 2020, the Food and Drug Administration (FDA) issued the authorization for emergency use and allowed the distribution of the Pfizer-BioNTech COVID -19 vaccine in the United States.

On 21 December 2020 the EMA (European Medicines Agency) issued a press release recommending conditional marketing authorization for the vaccine developed by BioNThec and Pfizer, to prevent COVID-19 in people 16 years of age and older. The scientific opinion of the EMA paved the way for the first authorization to place on the market a COVID-19 vaccine in the EU by the European Commission, with all the guarantees, controls and obligations that this implies. The AIFA (Italian Medicines Agency) also

authorized the marketing of the Pfizer-BioNTech vaccine on 22/12/2020 to be administered to subjects aged 16 or over and to be administered as a cycle of two injections.

On 18 December 2020, the FDA issued the authorization for emergency use (EUA) for the second vaccine for the prevention of COVID-19. The authorization allows the distribution of the Moderna COVID-19 vaccine in the United States for use in individuals aged 18 years or older. On 30 December 2020, The New England Journal of Medicine [55], analyzing data from approximately 30,000 participants enrolled in 100 clinical research centers, reported that the potential vaccine was found to be safe and well tolerated with an efficacy rate of 94.5%.

On January 29, 2021, the EMA, after careful evaluation of the quality, safety and efficacy of the AstraZeneca vaccine and Oxford University ChAdOx1 nCOV19 (AZD1222) vaccine with a non-replicating viral vector, recommended authorization for release to the market. This vaccine stands at an average efficacy level of 70%. In line with the decision of the EMA, on 30 of January 2021, the AIFA also authorized its use in Italy, underlining its preferential use in subjects between the ages of 18 and 55 for whom more solid evidence is available.

On 30 January–February 2021. AIFA recommended the AstraZeneca vaccine for over 55 s. Waiting to acquire further data from the studies currently underway for the Astra Zeneca vaccine, it suggested preferential use in populations for which there is more solid evidence, namely subjects between 18 and 55 years old. It also reiterated that, on the basis of immunogenicity results and safety data, the benefit/risk ratio of this vaccine is favorable even in older subjects who do not have specific risk factors. In March 2021 Germany limited the use of the Oxford-AstraZeneca COVID-19 vaccine to people 60 years of age and older due to concerns that it may be causing blood clots [63].

Vaccines without marketing authorization in Europe: SPUTNIK V—Gam-COVID—Vac of Gamaleya Res. Institute and Ministry of Health Russian Federation

On February 2, 2021, The Lancet magazine Logunov et al. [64] published ad interim results of the phase 3 study on Gam-COVID -Vac (SPUTNIK V) in which it is confirmed an efficacy against COVID 19 of 91.6% and good tolerance in a large cohort of patients. The randomized, double-blind, placebo-controlled study was carried out in 25 hospitals in Moscow and involved 21,977 adults randomly assigned to the vaccine group (n = 16,501) or the placebo group (n = 5476) with a 3:1 ratio. The vaccine comprises two vector components, rAd26-S and rAd5-S carrying the full-length gene for the S. SARS COV 2 glycoprotein, administered i.m. with an interval of 21 days between the first rAd26 dose and the second rAd5. The European Medicines Agency (EMA) has not received to

Table 2 Most common Antiviral drugs for COVID-19 and their mechanism of action

	Drugs	Target/mechanism of action
1	Favipiravir	Inhibits the RNA-dependent polymerase of SARS-CoV-2 by structurally resembling endogenous guanine
2	Ribavirin	Prodrug whose structure resembles adenosine
3	Remdesivir (GS-5734)	An inhibitor of the viral RNA-dependent, RNA polymerase
4	Galidesivir (BCX4430)	Adenosine analogue
5	Lopinavir/ritonavir	Protease inhibitors
6	Griffithsin	Targeting the S protein of SARS-CoV-2

date a cyclic review or marketing authorization application for SputnikV (Gam—COVID-Vac) vaccine developed by the Gamaleya National Center for Epidemiology and Microbiology in Russia.

The problem of variants: the English variant (Kent)

What is worrisome about variants is not where they come from, but the mutations they contain. Virus B.1.1.7 is characterized by a deletion in the spike protein and a mutation in N501Y that increases its transmissibility, as well as a potentially important mutation in the furin cleavage site. These mutations are set against the background of an unusually high number of other mutations that make B.1.1.7 distinct. In the case of B.1.351, the key mutation that makes it a threat to the effectiveness of a vaccine is the E484K mutation in the spike protein, also seen in P.1. The English variant was followed by the "South African" variant B.1.351 which contains a mutation reported to reduce the effectiveness of the ChAdIx Astra Zeneca vaccine, therefore South Africa is removing the vaccine from its vaccination schedule.

To summarize the current situation of COVID-19 vaccination, the following millstones and important dates are denoted. By 11 January 2020, the genetic sequence of SARS-CoV-2 was identified. By June 2020, dozens of vaccine candidates have prepared to prepare for global vaccination programs to immunize against COVID-19 infection [65]. On 24 June 2020, China approved the CanSino vaccine for limited use in the military, and two inactivated virus vaccines for emergency use in high-risk occupations. On 11 August 2020, Russia announced the approval of its Sputnik V vaccine for emergency use. The Pfizer–BioNTech partnership submitted an EUA request to the FDA for the mRNA vaccine BNT162b2 on 20 November 2020. As of 21 December, many countries and the European Union had authorized or approved the Pfizer–BioNTech COVID-19 vaccine. On 11 December 2020, the United States Food and Drug Administration (FDA) granted an Emergency Use Authorization (EUA) for the Pfizer–BioNTech

COVID-19 vaccine [66]. A week later, they granted an EUA for mRNA-1273, the Moderna vaccine. On March 31, 2021, the Russian government announced that they had registered the first COVID-19 vaccine for animals named Carnivac-Cov, it is an inactivated vaccine for carnivorous animals, including pets. In June 2021, a report revealed that the UB-612 vaccine, developed by the US-based COVAXX, was a venture initiated for profits by the Blackwater founder Erik Prince.

Conclusions

In this review, we provide a synopsis of genomic structure and evolution of SARS-CoV-2, and current efforts to produce effective vaccination and therapeutic strategies for SARS-CoV-2 infection. Most therapeutic strategies are based on repurposing of existing therapeutic agents used against various virus infections and focused mainly on inhibition of the virus replication cycle, enhancement of innate immunity, and alleviation of CRS caused by COVID-19. Currently, more than 100 clinical trials on COVID-19 aim to provide robust evidence on the efficacy of the currently available anti-SARS-CoV-2 antiviral substances, such as the nucleotide analogue remdesivir, the antimalarial drug chloroquine, and drugs directed against docking of SARS-CoV-2 to the membrane-associated angiotensin converting enzyme 2 (ACE2) such as transmembrane protease serine 2 (TMPRSS2) (Table 2). The current vaccination campaign is ongoing worldwide using different types of vaccines such as Pfizer-BioNTech and Moderna, Johnson & Johnson, Oxford-AstraZeneca, Novavax and others with an efficacy ranging from 72 to 95%. In March 2021 Germany limited the use of the Oxford-AstraZeneca COVID-19 vaccine to people 60 years of age and older due to concerns that it may be causing blood clots. Further study and more data are needed to confirm the safety of different available vaccines.

Declarations

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