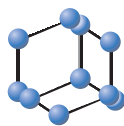


REVIEW ARTICLE


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Anti-Coronavirus Vaccines: Past Investigations on SARS-CoV-1 and MERS-CoV, the Approved Vaccines from BioNTech/Pfizer, Moderna, Oxford/AstraZeneca and others under Development Against SARS-CoV-2 Infection


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Abstract: The aim of this review article is to summarize the knowledge available to date on prophylaxis achievements in the frame of the fight against Coronaviruses. This work will give an overview of what is reported in the recent literature on vaccines (under investigation or already developed like BNT162b2, mRNA-1273, and ChAdOx1-S) effective against the most pathogenic Coronaviruses (SARS-CoV-1, MERS-CoV-1, and SARS-CoV-2), with of course particular attention paid to those under development or already in use to combat the current COVID-19 (CoronaVirus Disease 19) pandemic. Our main objective is to make a contribution to the comprehension, even at a molecular level, of what is currently ready for anti-SARS-CoV-2 prophylactic intervention, as well as to provide the reader with an overall picture of the most innovative approaches for the development of vaccines that could be of general utility in the fight against the most pathogenic Coronaviruses.

Keywords: Coronavirus, coronavirus infections, pandemics, COVID-19, HCoV, SARS-CoV, SARS-CoV-2, MERS-CoV, vaccine, BioNTech/Pfizer, Moderna, Oxford/AstraZeneca, BNT162b2, mRNA-1273, ChAdOx1-S, Vaxzevria.

1. INTRODUCTION

Coronaviruses (CoVs) are enveloped RNA viruses belonging to the family *Coronaviridae*, causing infections in birds, mammals and, among these, humans [1-6]. At the present time, seven human coronaviruses (HCoV) are known and described in the scientific literature [7], which are generally considered of zoonotic origin, and cause infections mainly associated with respiratory diseases [8-10].

On the one hand, HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1 are 'common cold'-associated coronaviruses provoking seasonal, usually mild, respiratory symptoms [11, 12]. Even though in most patients these HCoVs do not cause severe clinical

symptoms, HCoV-229E and HCoV-OC43 can provoke pneumonia [13, 14], while HCoV-NL63 and HCoV-HKU1 infections can also lead to bronchiolitis and croup [15, 16].

On the other hand, three highly pathogenic HCoVs emerged in the last two decades: Severe Acute Respiratory Syndrome (SARS)-CoV-1, Middle East Respiratory Syndrome (MERS)-CoV, and SARS-CoV-2 [17-19], leading in some cases to life-threatening pathologic events. Even though SARS-CoV-1 and MERS-CoV are more harmful in terms of lethality than SARS-CoV-2, the latter virus is more easily transmissible and this has led to the current pandemic of CoronaVirus Disease 19 (COVID-19), which is globally causing enormous problems in both health and socio-economic terms [20, 21].

The first step in coronavirus infection consists of a specific biomolecular recognition between the virus

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particle (the virion), through the CoV spike (S) protein, and the host cell, involving different HCoV-specific receptors [22-26]. These are known for several CoVs and are one of the primary targets for anti-CoV biomedical strategies [27]. The receptors associated with 'common cold'-HCoVs are human aminopeptidase N (APN), involved in the infection from HCoV-229E, and 9-O-acetylated sialic acid (9-O-Ac-Sia), associated with HCoV-OC43 and HCoV-HKU1. The receptor linked to HCoV-NL63, the angiotensin-converting enzyme 2 (ACE2), was found responsible for infections by the more pathogenic SARS-CoV-1 and SARS-CoV-2, while dipeptidyl peptidase 4 (DPP4) was associated with MERS-CoV [28, 29]. Once inside the cell, HCoVs replicate their RNA with a consequent expression of new viral proteins needed for the production of other viral particles inside the host organism [30]. While five out of the seven HCoVs are usually associated with mild upper respiratory infections, MERS-CoV and SARS-CoVs can lead to lethal events [31]. In particular, the new coronavirus SARS-CoV-2, first emerging in China at the end of 2019 [31], can provoke severe pneumonia and being easily transmissible it rapidly spread worldwide leading the World Health Organization (WHO) to declare COVID-19 a pandemic in March 2020 [32]. Currently, there have been more than two million deaths due to this pandemic (2,886,728 as found in Worldometers.info [33], accessed on 7th April 2021), with enormous consequences for public health and the global economy [34-36]. While the whole world is fighting against COVID-19 and waits for vaccines to be made available, the scientific community is devoting immense efforts to developing effective drugs for the immediate treatment of SARS-CoV-2 infection. Interestingly, several studies highlighted the role of lipid metabolism in COVID-19 [37] and suggested the lipid dysregulation as a drug target to contrast the pathological inflammation in patients [38, 39]. Nevertheless, due to the urgent need for effective pharmacological treatments, drug repurposing [40-42] and traditional medicine [43] are some of the most common approaches in this field with respect to new drug discovery [44].

2. PATHOGENIC HUMAN CORONAVIRUSES: SARS-CoV-1, SARS-CoV-2 AND MERS-CoV

The first recognized HCoV-associated pandemic, denominated as 'Severe Acute Respiratory Syndrome' (SARS), departed from China in 2002-2003 as a consequence of SARS-CoV-1 infection [45, 46]. Soon it spread to 29 countries worldwide, infecting more than

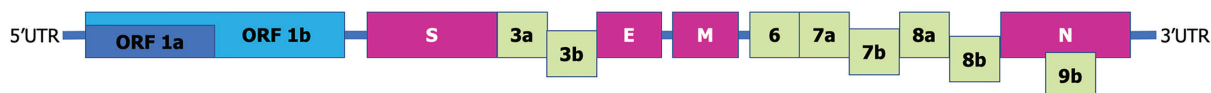
8000 people with a death rate of 10%. About ten years later, another deadly coronavirus emerged in the Middle East leading to the so-called 'Middle East Respiratory Syndrome' (MERS) and was consequently named MERS-CoV [47]. This outbreak took place among hospital workers in Jordan but, subsequently, it spread to other countries, including Saudi Arabia, where it was discovered in 2013-2014 [48]. Similar to SARS, MERS mainly affected adult individuals with a median age range of 39-50 years [14]. However, different from SARS, MERS predominantly affected male patients. Clinical symptoms common to both SARS and MERS are cough, fever, shortness of breath, and respiratory illness, but MERS was generally associated with a more severe pneumonia as well a higher mortality rate (up to 35% vs. 10% of SARS) [49].

SARS-CoV-2 is a HCoV closely related to SARS-CoV-1, sharing with it about 79% genome sequence identity, and is responsible, as already mentioned in this work, for the current COVID-19 pandemic [50]. Globally, a mortality rate of about 3.4% was estimated by WHO for the infection by SARS-CoV-2, even though this estimate did not find a definitive consensus [51]. Whatever the SARS-CoV-2 mortality rate, one of the main problems associated with this virus is its ability to spread easily and rapidly, which allowed it to reach in few months hundreds of countries worldwide, infecting more than 90 million individuals.

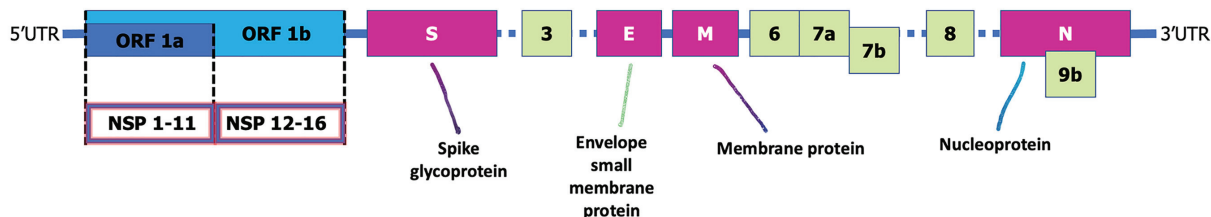
Owing to their classification, SARS-CoV-1, SARS-CoV-2 and MERS-CoV belong to the genus betacoronavirus. Moreover, SARS-CoV-1 and 2 are further classified into the *Sarbecovirus* subgenus, whilst MERS-CoV belongs to the *Merbecovirus* subgenus [52].

HCoVs are characterized by a single-stranded positive-sense RNA genome of about 30 kb. A schematic representation of the genomes of the three mentioned HCoVs is shown in Fig. (1). The structural S, E, and M proteins form the envelope of the virus, while the nucleocapsid used to pack genomic RNA is formed by the N protein. A particularly important role is associated with the S protein (spike glycoprotein) in virus-host cell receptor binding and virus-host cell membrane fusion in endosomes for the subsequent release of viral genome [53]. As previously mentioned in this work, both SARS-CoV-1 and SARS-CoV-2 utilize the host cell ACE2 receptor binding with the S protein, while MERS-CoV uses DPP4. At the cell surface, S protein is cleaved at the S1/S2 cleavage site into the domains S1 and S2, responsible for binding to the receptor and membrane fusion, respectively.

SARS-CoV-1



SARS-CoV-2



MERS-CoV



Fig. (1). Schematic representation and organization for the comparison of the viral genomes of SARS-CoV-1, SARS-CoV-2, and MERS-CoV, with a focus on SARS-CoV-2 proteins. The open reading frame 1ab (ORF1ab) is the largest gene, containing overlapping ORFs (indicated as two blue boxes) that encode polyproteins PP1ab and PP1a. The polyproteins are cleaved to obtain 16 non-structural proteins (NSP 1-16). Based on the similarity with other HCoV, these proteins include the papain-like proteinase protein (NSP3), 3C-like proteinase (NSP5), RNA-dependent RNA polymerase (NSP12, RdRp), helicase (NSP13, HEL), endoRNase (NSP15), 2'-O-Ribose-Methyltransferase (NSP16), and other non-structural proteins. The genes encoding for the structural proteins spike (S), envelope (E), membrane (M), and nucleocapsid (N) are shown as pink boxes. The genes encoding for accessory proteins, which are usually specific for each CoV genus and help in eluding host immune response, are in green. The main differences between the three genomes are highlighted by dotted lines. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

The first cleavage of spike glycoprotein, with subsequent separation of S1 and S2 domains, is mediated by human furin endoprotease. A second cleavage, mediated by transmembrane protease serine 2 (TMPRSS2), occurs at the S2' site of the S2 domain that is anchored to the membrane. This cleavage exposes the fusion peptide S2' with subsequent fusion of the membranes and viral genome entry into the cytosol of the host cell [54]. Moreover, it is speculated that SARS-CoV-2 possesses a unique furin-like cleavage site in the spike protein that may ensure a gain-of-function for the virus in entering the cells by promoting the furin-mediated S protein cleavage. This unique feature of SARS-CoV-2, which is not present in SARS-CoV-1, may explain the more efficient and rapid spread of this virus lineage compared to other HCoVs [55]. A scheme for the protein-protein interaction (PPI) network showing some virus-interacting human proteins was obtained by us within STRING database (Fig. 2) [56-58]. This used the interaction partners found by Gordon *et al.* [59] for the investigation of targets for drug repurposing against SARS-CoV-2, and other proteins involved in COVID-19 cell response to build the PPI (Fig. 2).

3. PROPHYLAXIS OF SARS AND MERS DISEASES

Notably, when SARS emerged in China in 2002, there were no vaccines or specific antiviral therapies available for combatting HCoVs. In fact, before 2002, the only known HCoVs were associated with 'common cold' symptoms; the majority of symptomatic patients usually recovered spontaneously without serious problems, so no drugs at all, or very few, such as aspirin, were administered to relieve the consequent cold-associated symptoms. Conversely, the critical clinical conditions often observed in patients affected by the more pathogenic MERS-CoV and SARS-CoV-1 showed the urgency of developing vaccines and antiviral treatments for HCoV infections. Nevertheless, the research on the theme of HCoV disease prophylaxis was probably not profound enough before the current pandemic emergency and, in our opinion, it should not be interrupted by scientists even if the COVID-19 pandemic is over.

In the case of SARS-CoV-1 infection, scientists undertook initial vaccine studies, but despite several can-

didates being included in clinical trials, none of them was approved by the US Food and Drug Administration (FDA) because of serious complications such as immune disease insurgence in treated animals [60]. In fact, a SARS-CoV-1 double-inactivated vaccine (DIV) performed poorly in animal models that displayed increased eosinophilic immune pathology in the lungs and resulted in no significant protection against HCoV replication. The research for other SARS vaccines was discontinued not only for the difficulties encountered, but mainly because SARS-CoV-1 vanished in 2004,

and thus, there was no interest to invest in the development of such vaccines [60]. Also, for MERS, despite several efforts in the search for effective vaccines, antibodies, and drugs, no conclusive results were achieved. In particular, this is because the development of a MERS vaccine had already encountered obstacles at the pre-clinical stages due to the lack of suitable and cost-effective animal models, and also because MERS incidence was sporadic and geographically restricted [61].

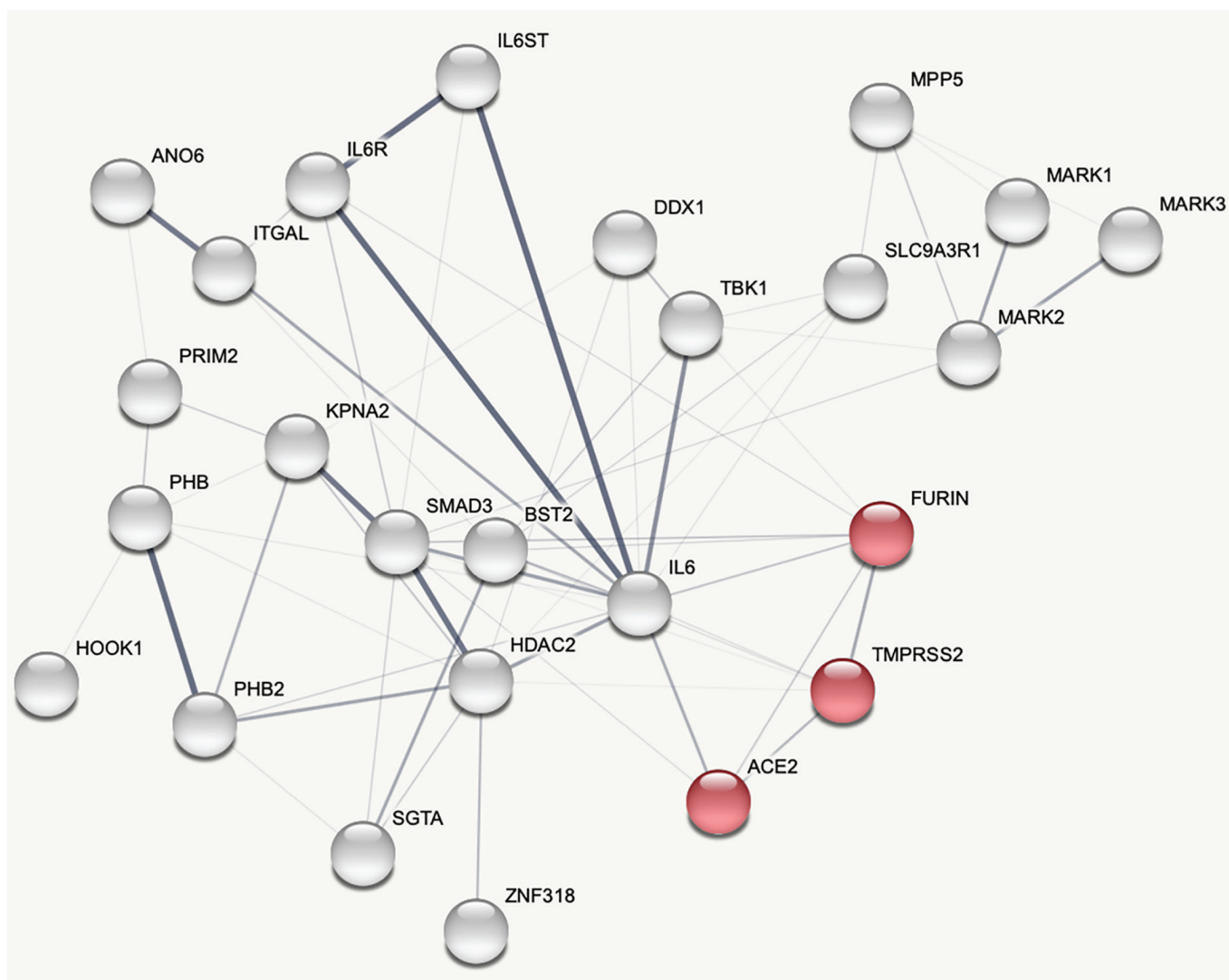


Fig. (2). PPI network of the SARS-CoV-2-interacting human proteins. The network reports the top 10 protein-protein interaction (PPI) partners found by Gordon *et al.* [59] with the addition of 15 proteins annotated in UniProt for being COVID-19-relevant (<https://string-db.org/cgi/covid.pl>). Using downstream STRING analysis, the main proteins involved in the first steps of viral-host cell infection are shown in red. (A higher resolution / colour version of this figure is available in the electronic copy of the article).

Table 1. Qualitative/quantitative review of the vaccine candidates developed and in use against SARS-CoV-2. Data based on COVID-19 vaccine tracker [66]. Last updated on 6th April 2021.

PLATFORM	TESTING VACCINES	VACCINES IN USE				
RNA	36	2	BNT162b2	mRNA-1273		
DNA	26	0				
Non-replicating viral vector	39	4	ChAdOx1-S	Ad5-nCoV	Gam-COVID-Vac/Sputnik V	Ad26.COVS.S
Replicating viral vector	24	0				
Inactivated	22	4	CoronaVac	WIBP vaccine	BBIBP-CorV	BBV152/Cov-axin
Live attenuated	3	0				
Protein subunit	100	2	EpiVacCorona	ZF2001		
Virus-like particle	23	0				
Other/Unknown	37	0				

The clinical trials for SARS and MERS vaccines were reviewed by Li *et al.* [62]. For SARS, one recombinant DNA plasmid vaccine named VRC-SRSD-NA015-00-VP by NIAID group and one inactivated virus vaccine named Inactivated SARS-CoV vaccine (ISCV) by Sinovac group only completed phase I. For MERS, one DNA vaccine named GLS-5300 (INO-4700) by GeneOne Life Science/Inovio Pharmaceuticals/International Vaccine Institute and one MVA (Modified Vaccinia virus Ankara)-based viral vector named MVA-MERS-S by CTC North GmbH & Co. KG also only completed phase I. The latter group produced an MVA vaccine (MVA-MERS-S_DF1) that did not complete phase I. Another two adenoviral-vectored vaccines for MERS, ChAdOx1 MERS by the University of Oxford and BVR5-GamVac by Gamaleya Research Institute of Epidemiology and Microbiology, are still in progress [62]. In particular, the ChAdOx1 MERS vaccine, a replication-deficient adenoviral-vectored vaccine expressing MERS-CoV S protein, protected rhesus macaques after a single intramuscular injection against MERS-related respiratory injury and pneumonia and significantly reduced viral load in lung tissue [63]. Similar protective effects were observed in dromedary camels [64]. Predictive studies were also conducted to designing novel multi epitope-based subunit vaccines by exploring MERS-CoV proteome [65], but there are no conclusive results on humans of such vaccines or others already explored like ChAdOx1 for the prophylaxis of MERS. Therefore, it appears possible that the lack of any useful vaccine against SARS-CoV-1 and MERS-CoV contributed to the current crisis, considering the relatively close genetic relationship between SARS-CoV-1 and SARS-CoV-2 as well as the conserved nature of MERS and SARS-CoV-2 proteins [45].

4. PROPHYLAXIS OF SARS-CoV-2 DISEASE

Even though there were no approved vaccines against the SARS-CoV-1 and MERS-CoV infections, the previous knowledge and attempts related to the targeted production of SARS and MERS vaccines have served rapidly to develop vaccines to be used against COVID-19. All the data reported regarding COVID-19 vaccines were reviewed by us interrogating the COVID-19 vaccine tracker platform [66]. As of April 6th, 2021, eight different technology platforms were under research and development. Even though the technology of numerous candidates remained undefined, most of those employed in clinical trials are based on the spike protein, and on nucleoside-modified mRNA and DNA. Hence, once delivered into the cell, the nucleic acids are translated into the spike protein itself as the primary antigen of SARS-CoV-2 able to induce the immune response. Other molecular platforms include replicating and non-replicating viral vectors, inactivated viruses, live attenuated viruses, virus-like particles, and recombinant proteins or peptides (Table 1) [67].

With the primary aim of saving time due to the magnitude and impact of the COVID-19 pandemic, many vaccine candidates have been directly submitted for clinical trials before pre-clinical data were acquired in animal models. Also, many vaccine examinations have been conducted adopting an integrated phase I/II or phase II/III approach for clinical trials [68, 69]. According to the COVID-19 vaccine tracker platform, among the 12 vaccines approved to be used worldwide, only four had published phase III results of their clinical trials and were ongoing phase IV. Two out of these four candidates are mRNA-based vaccines, while the other two are established with an adenovirus-based non-replicating viral vector technology (Table 2).

Table 2. Characteristics of the vaccines already in phase IV currently employed against SARS-CoV-2. Data based on COVID-19 vaccine tracker [66] and on the works of Polack *et al.* [70], Baden *et al.* [71], Voysey *et al.* [72] and Logunov *et al.* [73]. Last updated on 6th April 2021. Refrigeration (2°C to 8°C) is intended for up to 30 days, whereas frozen (-15°C to -25°C) for long-term storage.

VACCINE	BNT162b2	mRNA-1273	ChAdOx1-S	Gam-COVID-Vac/ Sputnik V
Developer(s)	BioNTech, Pfizer, Fosun Pharma	Moderna, NIAID	University of Oxford, AstraZeneca	Gamaleya Research Institute
Location(s)	USA, Argentina, Brazil, South Africa	USA	UK, Brazil, South Africa	Russia
Total participants	43,548	30,420	23,848	21,977
Platform	RNA	RNA	Non-replicating viral vector	Non-replicating viral vector
Dosing	2 doses, intramuscular	2 doses, intramuscular	2 doses, intramuscular	2 doses, intramuscular
Description	Lipid nanoparticle-formulated mRNA encoding full-length spike (S) protein	Lipid nanoparticle-encapsulated mRNA encoding pre-fusion spike (S) protein	Simian adenovirus vector containing codon-optimised spike (S) protein	Recombinant Ad26 (prime) and Ad5 (boost) viruses expressing full-length spike (S) protein
Published results	Phase I/II, phase III	Phase I, phase III	Phase I/II, phase II, phase III	Phase I/II, phase III
Estimated or validated efficacy	95%	94%	62-90%	92%
Age range (years)	≥16	≥18	≥18	≥18
Storage	Ultra-cold (-60°C to -80°C)	Refrigeration (2°C to 8°C) or frozen (-15°C to -25°C)	Refrigeration (2°C to 8°C)	Refrigeration (2°C to 8°C) or frozen formulation (maximum -18°C)
Manufacture projections (2021)	Up to 2 billion doses	Up to 1 billion doses	3 billion doses	No information on institute website
Approval status and distribution	Full or emergency use in numerous countries	Full or emergency use in numerous countries, including USA, Canada, EU, UK, and Israel	Emergency use in numerous countries, including UK, India, Argentina, Dominican Republic	Early or emergency use in numerous countries, including Russia, Belarus, Argentina, Serbia, and Algeria. Early or emergency use in Russia, Belarus, Argentina, and Serbia
REFERENCES	Polack <i>et al.</i> , NEJM 2020	Baden <i>et al.</i> , NEJM 2020	Voysey <i>et al.</i> , Lancet 2020	Logunov <i>et al.</i> , Lancet 2021

In detail, the two approved RNA-based vaccines were the BNT162b2, developed by the BioNTech/Pfizer/ Fosun Pharma group [70], and the mRNA-1273, developed by the Moderna/NIAID group [71], which showed an efficacy of 95% and 94% engaging around 43,000 and 30,000 participants, respectively [66]. No clinically approved examples of RNA vaccines were commercially available; thus, in the current scenario, the vaccines BNT162b2 and mRNA-1273 are the first ever approved RNA formulations for a mass vaccination. Focusing on RNA technology, RNA-based vaccines contain mRNA molecules that undergo translation into the human cells, producing antigenic viral proteins that once detected by immune cells are able to induce the immune response. Such formulations are

prepared as inclusions in lipid-based complexes (*i.e.* liposomes) that prevent RNA degradation and enhance the efficacy and the drug delivery [74]. RNA vaccines present many advantages, in fact they are configured to be safe, well-tolerated and highly adaptable to new pathogens. In addition, RNA formulations can be easily modified (they are called nucleoside-modified mRNA, modRNA) by synthetically replacing some nucleosides with others; thus, the desired protein is produced inside the cells, reconfiguring the conformational and structural properties of the native antigenic protein. Moreover, since the interaction between the RNA and the DNA of the host cell is thought to be unlikely, the risks of a possible genome integration seem to be suppressed. Nevertheless, within the scientific community

some voices are rising suggesting that regulatory safety assessments of mRNA vaccines, usually not classified as gene therapy products, should include genotoxicity studies [75, 76]. Some disadvantages are related to the possible risk of activation of the RNA-induced interferon response, which in turn would delay mRNA translation and promote its degradation inside the cells, nullifying the efficacy of the vaccine [77]. Also, some RNA vaccines require two-step mass vaccination and ultra-cold temperatures (from -20 to -80 °C) for storage and transportation, rendering their usage impractical mainly in developing countries.

The third candidate approved is an adenovirus-based non-replicating viral vector called ChAdOx1-S [72], and also known as Vaxzeveria, developed by University of Oxford/AstraZeneca, that until phase III showed a range of efficacy of 62-90% [66]. ChAdOx1-S is a simian adenovirus vector vaccine, which contains the information for the expression of the spike protein of SARS-CoV-2. Adenovirus technology-based vaccines consist of modified harmless viruses that act as delivery systems to present the antigens to the immune cells. Being properly modified, such vectors cannot replicate producing extra copies of themselves, thus ensuring increased safety. These vectors have a wide tissue tropism and induce a strong immune response, mimicking a natural infection. One approved example of adenovirus-based vaccine is that produced against Ebola virus [78]. Drawbacks of adenovirus-based technology include the risk of genomic integration and a diminished immunogenicity due to a pre-existing immunity within the human population. In recent years, the employment of simian derived-adenoviral vectors has circumvented the pre-existing human adenovirus immunity, with the neutralizing antibodies against such viral vectors being less frequent in the human population [79]. The second non-replicating viral vector candidate, the Gam-COVID-Vac/Sputnik V vaccine, was developed in Russia by the Gamaleya Research Institute. Gam-COVID-Vac is based on the combination of the recombinant replication-deficient adenoviruses type 26 (rAd26) and rAd5, which both carry the genes for the full-length glycoprotein S of SARS-CoV-2 (rAd26-S and rAd5-S). In this strategy, Ad26 is used as the “prime” and Ad5 as the “boost”. The main advantage of such kind of vaccine candidate is a more powerful and long-lasting immune response that can be achieved with the heterologous prime-boost vaccination approach. Data acquired on around 22,000 participants reported an efficacy for the Gam-COVID-Vac of 92%, configuring itself like one of the most efficient vaccines [73].

Other vaccine candidates were developed and experimented in their phase III clinical trials (Table 3). In this group, other two non-replicating viral vector have been included. The Ad5-nCoV developed by CanSino Biological Inc, Beijing Institute of Biotechnology [80] contains the full-length S protein sequence. For this vaccine phase I/II trial results were published. On the other hand, the Ad26.COVS vaccine produced by the Janssen Pharmaceutical Companies (Johnson & Johnson group) showed safety and immunogenicity profiles, and good efficacy after only one dose administered [81]. Thus, if further data on this vaccine candidate will be encouraging, its single dose administration could facilitate a rapid and efficient mass vaccination.

The remaining vaccine candidates are based on inactivated viruses or on protein subunit (Table 3). Inactivated virus vaccines have the benefit of inducing a broad range of humoral and cellular responses against all the different viral epitopes [87]. Previous practice on the elaboration of effective SARS vaccines has proved that the use of whole inactivated viruses can induce antibodies and protection against infection with SARS-CoV-1 but can also provoke short term adverse effects such as pulmonary immunopathology with eosinophil infiltration in animal models [88].

A whole inactivated virus-based vaccine consists of a non-active version of the target pathogen. The main advantage of such vaccines is that they are recognized by the immune cells, with subsequent induction of a strong immune response, but cannot be causative of illness. The vaccines against Hepatitis A, Influenza, and Rabies are examples of inactivated virus-based vaccines created to stimulate a proper response against such pathogens. Currently, several COVID-19 vaccines based on inactivated viruses are being developed [66]. As of 6th April 2021, four of them have already been approved for use despite the only published results for these vaccines being pre-clinical or clinical data in phase I/II, ongoing to phase III (Table 3). These vaccines are the CoronaVac (Sinovac) [82], WIBP vaccine (Wuhan Institute of Biological Products, Sinopharm) [83], BBIBP-CorV (Beijing Institute of Biological Products, Sinopharm) [84], BBV152/Covaxin (Bharat Biotech, ICMR, National Institute of Virology) [86].

CoronaVac is a patient-derived SARS-CoV-2 virus strain, which has been propagated in Vero cell line and inactivated with beta-propiolactone treatment. In the preclinical study, CoronaVac was shown to induce the formation of neutralizing antibodies against 10 representative SARS-CoV-2 strains in mice, rats, and non-

Table 3. Characteristics of the vaccines currently in phase II or III in use against SARS-CoV-2. Data based on COVID-19 vaccine tracker [66] and on the works cited in the table [73, 80-86]. Last updated on 6th April 2021.

VACCINE	Ad5-nCoV	Ad26.COV2.S	Corona Vac	WIBP vaccine	BBIBP-CorV	BBV152/Covaxin	EpiVac Corona	ZF2001
Developer(s)	CanSino Biological Inc, Beijing Institute of Biotechnology	Janssen Pharmaceutical companies	Sinovac	Wuhan Institute of Biological Products, Sinopharm001	Beijing Institute of Biological products, Sinopharm	Bharat Biotech, ICMR, National Institute of Virology	Vector Institute	AZLB, Chinese Academy of Sciences
Location(s)	China	Belgium, USA	China	China	China	India	Russia	China
Total participants	508	805	600	224	448	380	100	900
Platform	Non-replicating viral vector	Non-replicating viral vector	Inactivated	Inactivated	Inactivated	Inactivated	Protein subunit	Protein subunit
Dosing	1 dose, intramuscular	1 or 2 doses (to be determined), intramuscular	2 doses, intramuscular	2 doses, intramuscular	2 doses, intramuscular	2 doses, intramuscular	2 doses, intramuscular	2/3 doses, intramuscular
Description	Ad5 vector containing full-length spike (S) protein	Ad26 vector expressing stabilised pre-fusion spike (S) protein	Inactivated vaccine administered with aluminium hydroxide adjuvant	Inactivated vaccine adsorbed to 0.5-mg alum	Inactivated vaccine administered with aluminium hydroxide adjuvant	Inactivated vaccine administered with imidazoquinoline adjuvant (TLR7/8 agonist)	Peptide antigens conjugated to a carrier protein and administered with aluminium hydroxide	Adjuvanted recombinant protein vaccine targeting SARS-CoV-2 receptor binding domain (RBD)
Published results	Phase I, phase II	Phase I/II	Phase I/II	Phase I/II	Phase I/II	Phase I, phase II	None	Phase I/II
Estimated or validated efficacy	Pending	66%	Pending	Pending	79%	Pending	Pending	Pending
Age range (years)	≥18	18-35 ≥65	18-59	18-59	18-59	12-65	18-60	18-59
Storage	Pending - refrigeration (2°C to 8°C) or freezing (-15°C to -25°C)	Refrigeration (2°C to 8°C)	Refrigeration (2°C to 8°C)	Refrigeration (2°C to 8°C)	Refrigeration (2°C to 8°C)	Refrigeration (2°C to 8°C)	Pending - refrigeration (2°C to 8°C)	Pending - refrigeration (2°C to 8°C)
Manufacture projections (2021)	Pending	1 billion doses	No information on institute website	No information on institute website	No information on company website	No information on company website	5 million doses	Pending
Approval status and distribution	Full or emergency use in China, Mexico, and Pakistan	Early or emergency use in South Africa, Bahrain, and USA	Full or emergency use in numerous countries, including China, Azerbaijan, Brazil, Chile, and Colombia	Limited use in China and UAE	Full or emergency use in numerous countries, including China, UAE, Bahrain, Egypt, and Hungary	Emergency use in India, Iran, and Zimbabwe	Early use in Russia	Emergency use in China and Uzbekistan
REFERENCE	Zhu <i>et al.</i> , Lancet 2020	Sadoff <i>et al.</i> , NEJM 2021	Zhang <i>et al.</i> , Lancet Infect Dis 2020	Xia <i>et al.</i> , JAMA 2020	Xia <i>et al.</i> , Lancet Infect Dis 2020	Ella <i>et al.</i> , Lancet Infect Dis 2021	None	Yang <i>et al.</i> , Lancet Infect Dis 2020

human primates [62]. WIBP vaccine is a Wuhan patient-derived SARS-CoV-2 virus strain, which has been

propagated in Vero cell line and inactivated by two rounds of beta-propiolactone treatment. Phase I/II re-

sults showed that in all the patients that received the vaccine doses neutralizing antibodies were produced, and only a low frequency of unfavorable effects was recorded. A phase III clinical trial was launched in United Arab Emirates and Kuwait [62]. The BBIBP-CorV vaccine, being developed by the same company, Sinopharm, is very similar to the previous one, with the exception of the virus strain used. Again in this case, the phase I/II results showed the production of neutralizing antibodies; all the patients receiving the vaccine had an induced humoral response against SARS-CoV-2 42 days after immunization. Currently, in the United Arab Emirates and Argentina this vaccine is undergoing a phase III clinical trial [62].

Finally, the last two vaccines in use for COVID-19 prophylaxis are protein-based formulations. In particular, the one named EpiVacCorona has been developed by Vector Institute and its early use in Russia has been approved without undergoing further clinical trial experimentations, having involved only 100 volunteers for phase I/II testings. Phase III should be ongoing, with the inclusion of 3000 participants [66]. To date, this is the only vaccine constituted by antigenic peptides from SARS-CoV-2 to have been approved for use despite no published results being available in the medical literature. A second protein subunit vaccine, the ZF2001, was developed with the aim of targeting a dimeric form of the receptor binding domain (RBD) of the SARS-CoV-2 spike protein as antigen. The protein subunit vaccine ZF2001 emerged from results of the phase I and II to be well tolerated and immunogenic. These experimentations have supported the use of a three-dose schedule administration that will be further tested in a phase III trial for broaden evaluation of safety, immunogenicity, and efficacy of the ZF2001 vaccine [85].

CONCLUSION

The main lesson that the HCoV have taught us, with their impact on human life, is that both the worldwide scientific community and the pharmaceutical market should devote their efforts to develop vaccines against this very pathogenic family of RNA viruses without waiting for deadly outbreaks like the one we are witnessing. Fortunately, academic institutions and pharmaceutical companies have developed some promising vaccine candidates. Among them, those based on non-replicating viral vectors and RNA show very high efficacies giving the world hope that the recovery is closer. Mutagenicity and genotoxicity tests are advisable to exclude any potential adverse effects in this regard related to this new technology based on nucleic acids. In fact, the risk of genomic integration

cannot be excluded for vaccines containing viral DNA, and also those based on RNA. In this regard, several studies have demonstrated that eukaryotic cells exert, to some extent, reverse transcription activity [89-92], which could lead to DNA production theoretically starting also from the vaccine-delivered RNAs. Inactivated virus-based vaccines show efficacies higher than 50% but lower than those found in the above-cited cases. Moreover, previous practice on the elaboration of effective SARS-CoV-1 vaccines has proven that the use of whole inactivated viruses induces antibodies and protection against infection with SARS-CoV-1 but may also provoke adverse effects such as pulmonary immunopathology with eosinophil infiltration in animal models [88]. However, these adverse effects were observed at early stages in animal models (58 days after the administration with the specific vaccines) [88], while some inactivate virus-based anti-COVID-19 vaccines mentioned in this work entered advanced stages of clinical experimentation. On the other hand, there is obviously still no information available on possible long-term side effects related to any of the anti-SARS-CoV-2 vaccines already in use.

Another aspect that should be not ignored is that although the HCoV genomes are not as variable as other viruses, the receptor binding domain contained in the spike protein is the most mutable region, with mutation sites that lead to antibody escape variants [93-95].

In the particular case of SARS-CoV-2, various mutations have been detected involving the spike protein endowed in some cases with increased infectivity and reduced antibody binding [96]. Thus, it is probable that even in a context of mass immunization based on spike-targeting vaccines, other antibody-escaping mutations will emerge in the future making current anti-H-CoV protection less effective and vaccine development even more complicated. Therefore, vaccine candidates able to elicit a wider antibody repertoire together with a strong cellular immune response are clearly desirable as they may lead to a more durable and broader protection.

LIST OF ABBREVIATIONS

ACE2	=	Angiotensin-Converting Enzyme 2
APN	=	Aminopeptidase N
CoV	=	Coronavirus
COVID-19	=	Corona virus disease 19
DIV	=	Double-inactivated SARS-CoV-1
DNA	=	Deoxyribonucleic Acid

DPP4	= Dipeptidyl Peptidase 4
FDA	= Food and Drug Administration
HCoV	= Human Coronavirus
HEL	= Helicase
HIV	= Human Immunodeficiency Virus
ISCV	= Inactivated SARS-CoV Vaccine
MERS-CoV	= Middle East Respiratory Syndrome - Coronavirus
modRNA	= Nucleoside-Modified mRNA
mRNA	= Messenger RNA
MVA	= Modified Vaccinia Virus Ankara
NSP	= Non-Structural Proteins
9-O-Ac-Sia	= 9-O-acetylated sialic acid
ORF	= Open Reading Frame
PPI	= Protein-Protein Interaction
RdRp	= RNA-dependent RNA Polymerase
RNA	= RiboNucleic Acid
SARS-CoV	= Severe Acute Respiratory Syndrome - Coronavirus
STRING	= Search Tool for the Retrieval of Interacting Genes/Proteins
TMPRSS2	= Transmembrane Protease Serine 2
UTR	= Untranslated Region
WHO	= World Health Organization

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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