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RESEARCH ARTICLE

Mapping the technological landscape of SARS, MERS, and SARS-CoV-2 vaccines

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ABSTRACT

Purpose: The last two decades have seen the emergence of several viral outbreaks. Some of them are the severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and severe acute respiratory syndrome 2 (SARS-CoV2) – the cause of the coronavirus disease 2019 (COVID-19). Ever, vaccines for emergency use have been authorized for the control and prevention of COVID-19. Currently, there is an urgent need to develop a vaccine for prophylaxis of COVID-19 and for other future epidemics.

Methods: This review describes patented vaccines for SARS and MERS-CoV and vaccines developed and approved for emergency use against the new coronavirus (COVID-19). The European Patent Office and the World Intellectual Property Organization were the patent databases used using specific terms. In addition, another search was carried out in the Clinical Trials in search of ongoing clinical studies focused on the COVID-19 vaccine.

Results: The patent search showed that most vaccines are based on viral vector platforms, nucleic acids, or protein subunits. The review also includes an overview of completed and ongoing clinical trials for SARS-CoV-2 in several countries.

Conclusion: The information provided here lists vaccines for other types of coronavirus that have been used in the development of vaccines for COVID-19.

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Introduction

Vaccination is one of the most effective strategies to save lives in the medicine history [1]. The technology of the classical vaccines is based on the use of killed or attenuated pathogens, which expose the body to antigens but cannot cause the disease, and elicit an immune response able to respond to the disease of contact with pathogens [2]. Although vaccines have the same action mechanism, there are eight main types classified according to their composition: inactivated or attenuated virus, replicating and non-replicating viral vector, based on RNA or DNA, protein subunit or virus like particle [3,4].

Severe acute respiratory syndrome 2 (SARS-CoV-2) emerged in China in November 2019 and spread rapidly to become a worldwide pandemic [5,6]. The disease caused by the virus was given the name coronavirus 2019 (COVID-19), and triggered the urgent development of vaccines against it [7,8]. Previously, two other related viruses of the same genera (betacoronavirus) have been involved in human outbreaks of acute respiratory syndrome. The first appearance in Southern China in 2003 was known as SARS-CoV, and almost a decade later, the Middle East respiratory syndrome (MERS-CoV) emerged in Saudi Arabia [9–11].

At the beginning of the pandemic, the lack of knowledge about the pathology and physiology of the new coronavirus, in addition to the virus's ability to mutate and recombine, contributed to the absence of licensed vaccines when the virus spread around the world [12–15]. Currently, with the start of the global vaccination against SARS-CoV-2, the situation may improve concerning the number of deaths and infections [16].

In this way, several countries have already requested the emergency use of vaccines produced with different platforms as an immunization strategy to contain the pandemic's advances. Despite the proof of the efficacy and safety of approved immunizers, limitations involving economic, logistical and transportation issues arise this long. Each country's governors must establish logistical details to guarantee the population's immunization process's effectiveness and efficiency. Thus, there must be strategic planning regarding the methods of transport, storage, handling, control and distribution of vaccines [12,13].

Until February 2020, vaccines that have been developed against the COVID-19 are either not effective, or in some situations have been related to being involved in the choice of novel pathogenic CoVs by recombination of circulating strains [17]. Research on vaccines' development increases and includes protein subunit vaccine platforms, such as Spike protein antigens of SARS-CoV-2, that target the virus's receptor-binding domain; DNA and RNA vaccines using genomic sequence information. In addition to viral vector (replicating and non-replicating), inactivated or live attenuated virus, virus-like particle and viral vector with the antigen-presenting cell [8,18–22].

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The transmission of CoVs can happen across species, through person-to-person contact, the aerosol transmission of respiratory droplets emitted coughing or sneezing, and by touching contaminated surfaces [23,24]. Due to the speed of the transmission of the virus during the pandemic, the initial main targets of any vaccine developed should be healthcare workers, patients with the disease, and those who have been in contact with them [25].

Currently, according to data from the World Health Organization (WHO), the global COVID-19 vaccine research and development (R&D) landscape for the new pandemic outbreak includes 251 vaccine candidates, of which 181 are in pre-clinical development and 70 in clinical development (it is sometimes difficult to assess the actual current status of development due to a lack of publicly available information on proprietary products) (updated WHO, 19 February 2021). One critical point that causes concern, especially for those who urgently need these vaccines, is that industry benchmarks for traditional vaccine development paradigms cite attrition rates for licensed vaccines of more than 90%. However, these benchmarks may not be applicable in a pandemic that has changed paradigms, as in the case of the COVID-19 outbreak [26,27].

This review aims to evaluate patents that have developed vaccines to prevent SARS-CoV-1 and MERS-CoV, and provide an update on the worldwide vaccine landscape in a clinical study for COVID-19.

Methods

In our review, the patents cover the period from 2005 to 2020, the European Patent Office (EPO) and World Intellectual Property Organization (WIPO) databases were searched using the descriptor 'coronavirus and MERS,' 'coronavirus and COVID' and 'coronavirus and SARS' in the title and abstract. A total of 402 patents were identified for preliminary assessment from the databases, of which 225 patents were excluded as they were duplicates, 10 because the text was unavailable, 150 for being outside the focus (vaccine) of our review after reading the title and abstract, and a further three patents were excluded after reading the full patent for being outside the focus of the review. This resulted in 14 patents being selected for our critical analysis according to the objective of the study. Figure 1 illustrates the systematic guidelines, based on the PRISMA methodology, used for the patent search and screening in this review.

In order to assess the progress of clinical trials aimed at developing a vaccine for SARS-CoV-2, an advanced search was conducted using the website of the Clinical Trials. The condition or disease fields 'SARS-COV-2 or COVID-19' and intervention/treatment 'vaccine' were used, resulting in the identification of 211 ongoing studies (updated, 12 January 2021). Of these, 119 trials were selected, and 92 were excluded because they were not related to a new COVID-19 vaccine. Factors such as the evolution

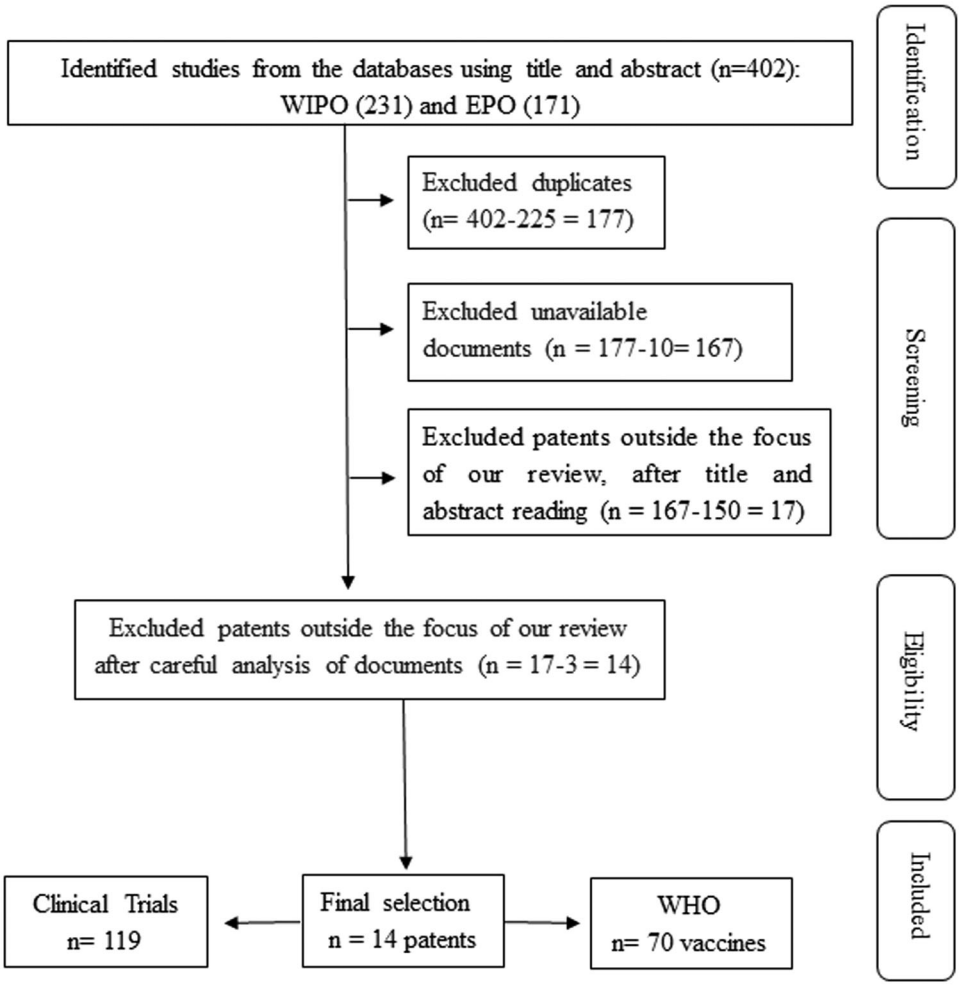

Figure 1. Flowchart of patent search and screening.

of the number of studies over time, the clinical phase of the study, recruitment status, sample size, geo-distribution of clinical trials, and source of financing were mapped.

In order to obtain more up-to-date data on the development situation of the candidate vaccines against COVID-19, the WHO website was used as a reference [27]. Thus, we sought to compare WHO data with information obtained from patent documents and clinical studies in progress.

Besides, one of the databases used to search for patents in this review (Web of Science) adds metadata to patents as part of its Derwent Innovations Index. This allows the data to be searched many of different ways, including searching for terms associated with the patents [28]. Therefore, we decided to conduct a bibliometric cluster analysis of these terms in coronavirus-related patents to see if this could help provide a clearer picture of previous research in this area. A search was carried out in the database with the keywords 'coronavirus and COVID-19' (6), 'coronavirus and MERS' (156), 'coronavirus and SARS' (699), 'coronavirus and SARS-CoV-2' (5), and 'coronavirus and 2019-nCoV' (13). A total of 879 patents were identified with 17,538 related terms. We then selected the terms that were mentioned 20 or more terms, which resulted in 280 items. We used the database tools to calculate a relevance score and selected items with strong relevance. Of the 168 terms identified in this way, 126 terms were excluded using a manual search, as they did not meet the search criteria or were not relevant. Finally, a co-occurrence map composed of 42 terms was obtained.

Results and discussion

Patent search and screening

The first patent found was published in 2005, which includes the emergence of SARS-CoV-1 in Asia in 2003, when the virus is spreading worldwide, infecting and killing thousands of people. Although the number of patents increased between 2005 and 2008 (Figure 2(A)), these values decreased as the scientific community realized the low prevalence and transmissibility of MERS-CoV. The patent office with the most filings was the World Intellectual Property Organization (WIPO) with six patents, followed by China (CN) with four patents, and the European Patent Office (EP) with two patents (Figure 2(B)).

Different scientific entities may request the protection of an invention using a patent application. In this respect, the industry led the filing ranking with 12 patents (Figure 2(C)). Applicants include GlaxoSmithKline Biologicals S.A., Institute Pasteur, Curevac AG, the Tumor Prevention & Treatment Center, Bio-Pharm Science & Technology Co., Ltd, and ID Biomedical Corporation. Also, the following universities also established partnerships with these companies for product development in the case of four patents: Tsinghua University, Johns Hopkins University, Ludwig-Maximilians-Universität München and Philipps-Universität Marburg. Independent researchers can patent their inventions without establishing partnerships with companies (3) and or universities (2), and represent a total of five patents.

In respect of the areas of the patents, the International Patent Classification (IPC) represents a hierarchical system for classifying patents based on the technological areas to which they belong. In this review, the most frequent were the IPCs A61K, which appears in 12 patents (Figure 2(D)) and refers to 'preparations for medical, dental, or toilet purposes,' C07K with one patent that refers to 'peptides,' and C07H with one patent that refers to 'sugars; derivatives thereof; nucleosides; nucleotides.'

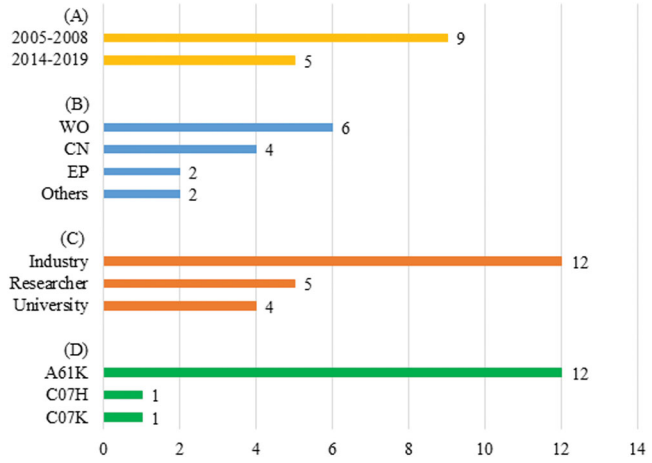

Figure 2. Number of patents. (A) Publication date. (B) Country according to the Patent Publication Location – WO: World Intellectual Patent Organization; CN: China; EP: European Patent Office. (C) Patent applicant. (D) International Patent Class (IPC) – A61K: Preparations for medical, dental, or toilet purposes; C07H: Sugars; derivatives thereof; nucleosides; nucleotides; C07K: Peptides.

Clinical trial search and screening

Our results show that 119 clinical studies are being conducted by industry/private developers, academic, public sector and nonprofit organization using different platforms [29]. It is important to note that the search for vaccines for COVID-19, so far, is focused on symptom control and not on infection protection. It is an important strategy to be used considering that along with the global health system crisis, the economy is also affected, so this strategy can provide hope of restoring normality [30].

The appearance of a new outbreak is of great concern to health entities, especially when it becomes a pandemic. In the case of SARS-CoV-2, the race for the search for vaccines has accelerated the traditional process for developing a vaccine that usually takes years to go through the clinical phases with humans to evaluate efficacy and safety. Developing a vaccine for a pandemic is a financial risk for companies, as they do not know if the product will be found to be safe and effective and go on to be manufactured. Preparations may include making provision for production on an industrial scale, even before confirmation of clinical results and the promise of returning on their investment [31,32]. For all the reasons explained here, developing a safe, effective, and viable vaccine for the world population is complex and expensive [33].

Figure 3(A) shows that in the first half of 2020, clinical trials began to be registered, at which point the strong need to create and evaluate a vaccine for COVID-19 was realized due to the scale of the outbreak [34]. In the second half, this number doubled, months after the WHO characterized COVID-19 as a pandemic. Many number of entities began to conduct more clinical studies due to the increase in the number of infected and dead worldwide [35,36]. About 13 other studies are scheduled to be carried out in several countries from January to March 2021 to assess the efficacy and safety of available vaccine candidates.

Clinical trials have three phases (I, II, III) that seek to evaluate a safe dosage range and possible side effects. They start with a small group of healthy individuals before moving on to a larger sample of individuals. They are tested on infected individuals from different regions and countries before commercialization, which corresponds to Phase IV [37]. According to the data found, 29 of the studies are in phase 1 that evaluates vaccine safety and dosage in a small group of volunteers. In comparison, about 32

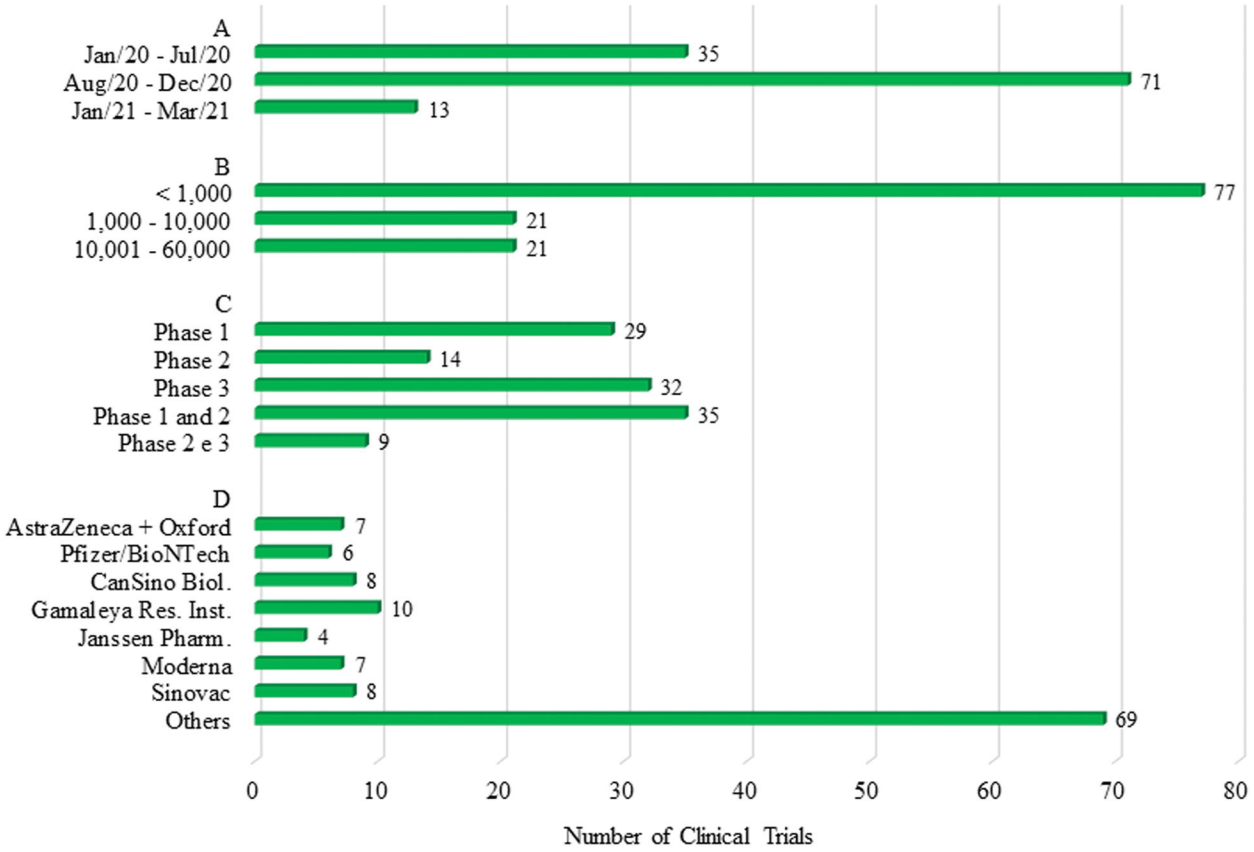

Figure 3. Number of clinical trials of for SARS-CoV-2's vaccines: (A) first registered; (B) vaccinated patients; (C) current clinical phase; (D) leading vaccine developers.

studies are in phase 3 to assess immunizers' effectiveness on a large scale. The sample size of these volunteers ranges from 1,000 to 60,000 individuals (Figure 3(B,C)).

As expected, varieties of candidates are needed to immunize a portion of the world's population successfully. In this way, several countries have conducted clinical studies in their population, intending to evaluate the vaccines' safety and efficacy. Figure 3(D) shows the main developers of the most promising vaccines that have been tested in several countries. The vaccines developed by companies such as AstraZeneca and Oxford University, Pfizer and BioNTech, CanSino, Moderna, Janssen Pharmaceuticals, Gamaleya Research Institute, and Sinovac are of the greatest interest of different governments due to the efficacy and safety offered by vaccines.

Concerning the geo-distribution of clinical studies, the vast majority are concentrated in the United States, China and Russia and Canada. Such fact is attributed that the companies that develop the promising vaccines are headquartered in these countries. Even so, another 44 countries located on the five continents also have clinical studies in progress. In this way, a portion of the population in different countries with different mutations in SARS-CoV-2 will be immunized against the virus (Figure 4).

As expected, 100 ongoing clinical studies count on the participation and financing of companies from the private or public sector, and universities and the formation of partnerships. Of these studies, about 50% are in recruitment status, 46% are not yet recruiting volunteers, and 4% have completed their studies (Figure 4).

The current trials of vaccines developed to fight the SARS-CoV-2 infection are distributed across several stages of the process. A large portion is still performing pre-clinical studies in animals to verify the production of an immune response. However, others have already administered a vaccine to a small group of healthy volunteers to assess safety and dosage issues. Hundreds of people are now involved in these trials, with vaccines being tested in different groups, such as older adults and children, to assess their safety and the generation of an immune response [38]. Now, in the final stage of the trial, thousands of people have been tested to verify each vaccine's effectiveness. Table 1 (updated WHO, 19 February 2021) shows the most promising vaccines, their effectiveness, number of doses, interval, and countries that have already started immunization.

Cluster analysis

The bibliometric analysis showed that the five terms with the highest frequency of co-occurrence were: syndrome (416), protein (243), sequence (199), antibody (180), vaccine (148). These terms represent the patents' main content, and their frequency of occurrence and co-occurrence reflects research in particular areas. We used the VOSviewer software to produce a co-occurrence map. Its shows that the selected keywords were divided into three groups, represented by different colors. The software seeks to maintain terms of similar areas of interest in the same cluster [28]. Detailed information on each cluster is presented in Table 2. The terms present in the clusters reflect the focus of research on the areas.

The cluster map based on co-occurrence expresses the frequency of terms according to the node's size, and the thickness of the line represents the proximity of the connection between the keywords [28]. Therefore, according to Figure 5, syndrome, protein, sequence, and vaccine are the most frequent. The thick connecting lines between the terms, syndrome, vaccine, protein, sequence show that they have a strong connection. Similarly, the terms, vector, antibody, adenovirus, immunogenic composition,

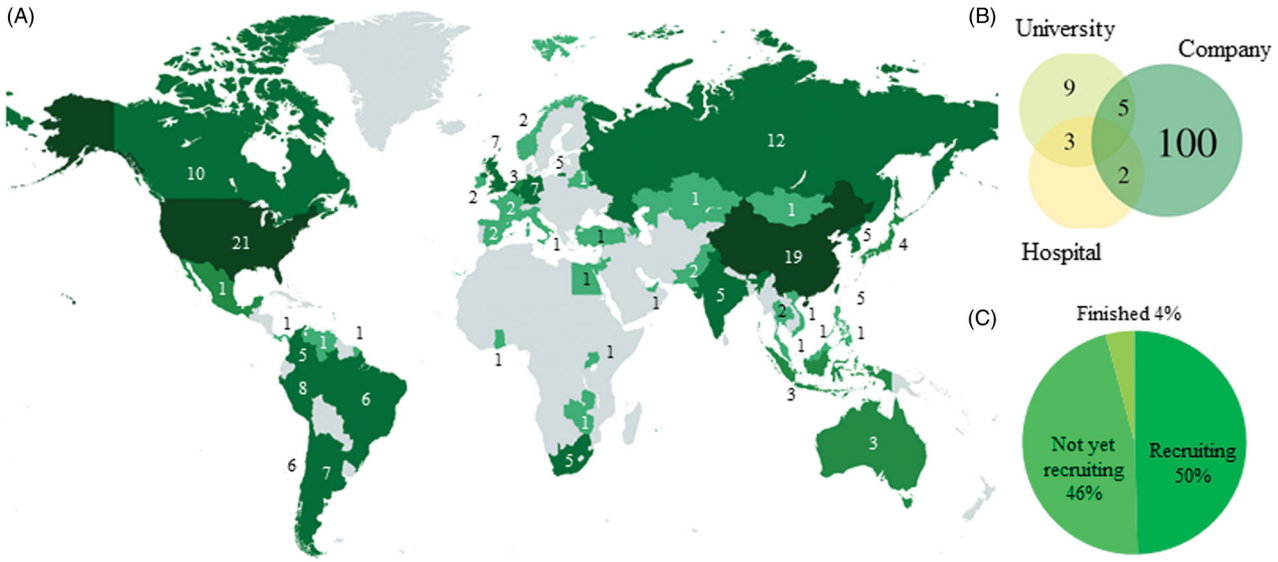

Figure 4. Number of clinical trials for SARS-CoV-2's vaccines: (A) distribution of clinical trials in each developer country; (B) distribution of clinical trials by type of sponsor; (C) recruiting status.

Table 1. Leading vaccines for COVID-19 (Updated Feb. 19, 2021).

Vaccine name	Developers	Efficacy	Phase	Dose	Approved or emergency use
Comirnaty	Pfizer; BioNTech	95%	Phase 2/3	2 doses, 3 weeks apart	Argentina, Canada, Chile, Costa Rica, Ecuador, Jordan, Kuwait, Mexico, Panama, Singapore, Bahrain, Saudi Arabia, Switzerland, European Union
mRNA-1273	Moderna; National Institutes of Health	94.5%	Phase 3	2 doses, 4 weeks apart	United States of America, Canada, Israel and European Union
Sputnik V	Gamaleya Research Institute; Health Ministry of the Russian Federation	91.4%	Phase 3	2 doses, 3 weeks apart	Russia, Bolivia, Argentina, Algeria, Serbia, Belarus.
AZD1222	University of Oxford; AstraZeneca	62% to 90%	Phase 2/3	2 doses, 4 weeks apart	Argentina, India, Morocco, Britain, Mexico, El Salvador, Dominican Republic
BBIBP-CorV	Sinopharm	79.34%	Phase 3	2 doses, 3 weeks apart	China, Jordan, Egypt, Bahrain, United Arab Emirates
CoronaVac	Sinovac	< 78%	Phase 3	2 doses, 2 weeks apart	China
Convidecia	CanSino	Unknown	Phase 3	Single dose	China
Ad26.COV2.S	Johnson & Johnson	Unknown	Phase 3	Single dose	
EpiVacCorona	Vector Institute	Unknown	Phase 3	2 doses, 3 weeks apart	Russia
NVX-CoV2373	Novavax	Unknown	Phase 3	2 doses, 3 weeks apart	
	Sinopharm-Wuhan	Unknown	Phase 3	Unknown	China; United Arab Emirates
Covaxin	Bharat Biotech	Unknown	Phase 3	2 doses, 4 weeks apart	India

Source: Zimmer, Corum and Wee [38].

have a weak connection. This bibliometric analysis supported the findings of our main analysis that research in vaccines revolves around the use of virus proteins – developing a sequence of amino acids for use in vaccines; stimulation of immune response with recruitment of antibodies; and the use of viral vectors.

Coronavirus vaccine patents

Studies on vaccines should provide information on their main protective activities about provoking immune responses triggered by T and B cells, and the virus's main antigens, and its molecular biology and pathogenesis [39]. Vaccines produce different immune responses depending on the type of vaccine and the pharmaceutical supplies present in the vaccine. Live vaccines generate high cellular responses and provide lifelong protection, requiring more than one dose. On the other hand, inactivated vaccines, toxoids or subunits require booster doses to achieve similar results. In addition, responses also vary by composition. The use of polysaccharides as adjuvants induces an independent T cell response, not generating an immune response and presenting a short life [40].

The immune response directed to the pathogen can occur initially through the innate nonspecific response, followed by the appearance of a specific adaptive response. While the innate immune response appears soon after infection, the adaptive response develops for weeks, seeking to generate a lasting immune memory. In addition, $CD4^+$ and $CD8^+$ effector T cells are recruited. $CD4^+$ cells act by mediating the activation of macrophages by releasing cytokines so that pathogen destruction occurs. Also, they help B cells to produce high-affinity antibodies.

$CD8^+$ cells release cytotoxic factors to eliminate cells infected with pathogens [41,42].

In the current situation of the COVID-19 pandemic, 70 vaccine candidates are in clinical development worldwide. Approximately 33% of these used protein subunits of the virus as their approach, 16% non-replicating viral vector and 14% inactivated virus. About the number of doses and intervals of administration, about 60% of the candidates have two doses, applied between day 0 and day 28 (29%) [27].

The patents identified in this review relate to coronavirus vaccines that are viral (28.6% – four), protein subunit vaccines (35.7% – five), and nucleic acid vaccines (35.7% – five) (Table 3). All viral vaccines express at least one protein of the pathogen, either a spike (S), membrane (M), or envelope (E) protein.

Viral vaccines

Of the four patents using viral vaccines, three are based on modified adenovirus and the other on modified vaccinia virus Ankara (MVA), a highly attenuated and replication-deficient strain vaccinia virus. All virus-based strategies were able to elicit a specific antibody immune response in animal models. The advantage of viral vaccines lies in their capacity to mimic the natural virus infection and elicit high antibody and cellular responses. Due to their high immunogenicity, the use of vaccinal adjuvants is often not required, thereby reducing any adverse effects. However, viral-based vaccines have some weaknesses, the most important being: the possibility of reversion to virulent forms, the necessity of cold chain equipment for transport and storage, and the development of vector-neutralizing antibodies after the first immunization [57,58].

Table 2. Patent clusters on vaccines.

Cluster	Number of keywords	Keywords
1	24	Acid sequence, amino acid sequence, antibody, bind, binding, expression. Vector, fragment, host cell, immunogenic composition, mers cov, monoclonal antibody, nucleic acid, nucleic acid molecule, nucleic acid sequence, peptide, polypeptide, protein, sars cov, sars cov infection, sequence, spike protein, vaccine, vaccine composition, vector
2	14	Adenovirus, echovirus, enterovirus, *Flaviviridae*, hantavirus, *Picornaviridae*, poliovirus, *Retroviridae*, rhinovirus, rotavirus, simplex virus syndrome, *Togaviridae*, vaccinia virus
3	4	DNA, nucleotide sequence, polynucleotide, RNA

Of these patented vaccines, two used adenovirus as a vector for immunization against SARS-CoV-1. The vaccine developed by Huang *et al.* [53] was able to elicit a specific antibody immune response *in vivo* using an adenovirus type 5 (Ad5). The use of adenovirus facilitates the infection of the respiratory mucosa epithelium, and the induction of the respiratory mucosa immune response and prevention of humoral immunity. To development the vaccine, serum from infected and recovered individuals were collected and total SARS-CoV RNA was obtained by isolation, isolating the S gene. Subsequently, this gene was cloned into plasmid pShuttle and inserted into the adenovirus structure plasmid (pAdeno-X^{TM}).

The present invention presented high safety and convenience in its use, observed in *in vivo* expression test using the vaccine by intramuscular and intranasal use. Rodents belonging to the treatment group received 0.5 ml of intranasal drip of the vaccine, administered once a week for three consecutive weeks. After the first administration, blood was collected and two weeks after the last dose, the animals were sacrificed and blood and tissues were collected. Thus, IgG Anti-SARS levels were measured and showed high levels of antibodies with the negative control (without treatment). Similarly, the viral vector vaccine containing adenovirus capable of expressing the S, M, and E proteins of SARS-CoV-1 promoted a humoral immune response in mice after four weeks of vaccination [54].

In addition, Zhang *et al.* [56] also developed an adenovirus-based vaccine against MERS-CoV that produced increased levels of specific antibodies after nasal and intramuscular administration. However, this invention uses a chimpanzee adenovirus vector AdC68, avoiding the preexisting immunity in the Ad5 vector. Although, it maintains the advantages of the method as easy production and storage. *In vivo* tests performed on BALB/c mice evaluated the administration of 20 µl of nasal drip and 50 µl intramuscularly of the vaccine in a single immunization. Blood samples were collected from the second week after application of the vaccine and every two weeks. Thus, the levels of total IgG antibodies induced by the vaccine were detected from the collected samples. All groups tested with nasal and intramuscular administration in different concentrations produced high levels of

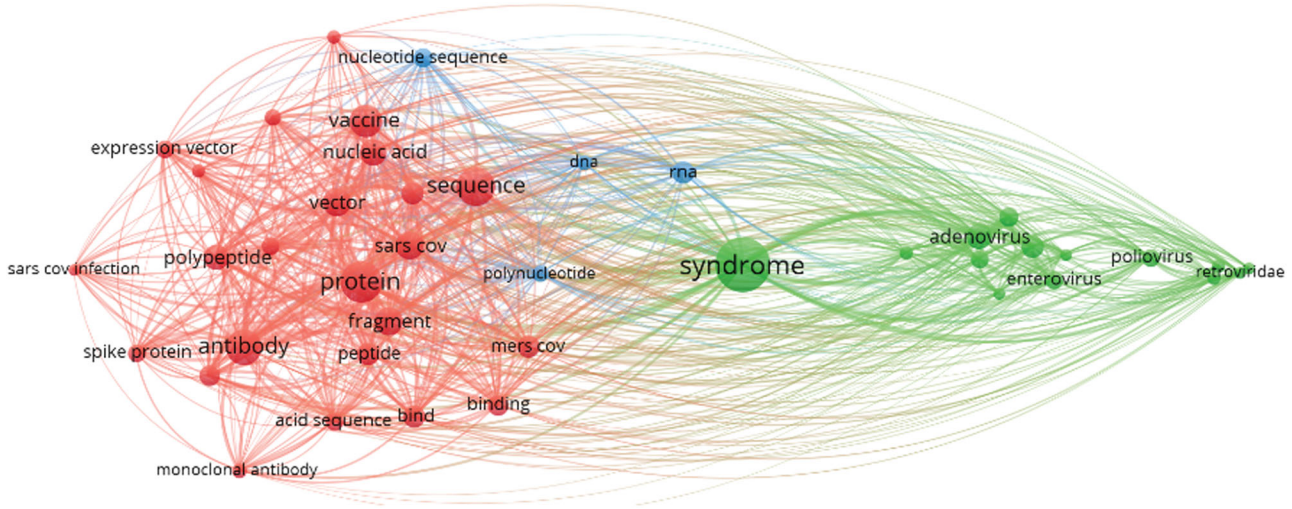

Figure 5. Clusters with correlation of the main keywords. Made by the author using the software VOSviewer.

Table 3. SARS and MERS coronavirus vaccine patents.

Author	Year	Country	Description	Vaccine development mechanism	Assay *in vivo*
Wu et al. [43]	2005	WO/US	DNA vaccine encoding N SARS-CoV-1 protein with antigen linked to CRT.	Nucleic Acid	Antibodies and cell mediated immune response in animals after vaccine administration. *In vivo* protection after challenge model
Zeng et al. [44]	2005	CN	Nucleic acid vaccine to prevent SARS-CoV-1 based on DNA vaccine expressing the gene S of SARS-CoV-1.	Nucleic Acid	Specific immune antibodies response in rats after three-dose immunization protocol.
Shao et al. [45]	2007	WO/CN	Vaccine contains replication-type vaccinia virus as a vector encoding the NC protein and the protruding protein of SARS-CoV-1 as a DNA vaccine strategy.	Nucleic acid	Induction of the production of high levels of cellular and antibodies response after mice immunizations.
Weiner et al. [46]	2015	WO/US	MERS-S or MERS-S-ACD DNA vaccine based on Spike protein.	Nucleic Acid	$CD8^+$ and $CD4^+$ T cells responses with production of IFN-γ, TNF-α and IL-2 in immunized mice. Neutralization activity of the produced antibodies.
Rauch [47]	2018	WO/DE	mRNA-based vaccine containing at least one spike (S), S1 spike (S1), membrane (M), nucleocapsid (N) of MERS-CoV.	Nucleic Acid	Induction of humoral immune responses after immunization. Evaluation of efficacy and safety has started clinical trial (phase 1).
Enjuanes and Almazan [48]	2006	WO/ES	Nucleic acid-containing vaccine encodes SARS-CoV replicase, and protein N, M, E, S.	Protein subunit	NA
Burt et al. [49]	2007	EP/US	Vaccine comprising immunogenic protein S and an adjuvant such as Protollin™	Protein subunit	High IgG levels in intranasally immunized mice capable of eliciting a protective immune response.
Jiang et al. [50]	2007	EP/US	Vaccine comprising a RBD domain of spike protein fused to Fc region of mouse IgG1.	Protein subunit	High antibodies titers after subcutaneous or intradermal mice and rabbit immunization. The elicited immune response could neutralize the infection in cell culture
Baras et al. [51]	2008	WO/FR	Vaccine composed of immunogenic polypeptide of the SARS-CoV-1 protein S and adjuvants.	Protein subunit	Histopathology showed no specific lesion of alveolitis or pneumonia in the lungs of rats and hamsters vaccinated with vaccine and adjuvants. Induction of $CD4^+$ T cell response and cytokine production in mice. Production of type Th1 (T helper 1) cytokines.
Tan et al. [52]	2014	CN	Synthetic peptide vaccine for MERS-CoV based on Spike protein parts (SP2, SP3 and RBD).	Protein subunit	Immunized animals produced high levels of antibody-specific titers. Neutralization capacity of immune serum SP3 (MERS) and protein SBD (SARS).
Huang et al. [53]	2005	CN	Adenovirus defective vector SARS-CoV-1 vaccine.	Viral vector	Specific immune antibodies response in rats after three-dose immunization protocol.
Zhou and Dong [54]	2005	WO/CN	Vaccine containing the recombinant adenovirus vector that expresses protein S, M and E	Viral vector	Humoral immune response in the mice after fourth week of vaccination protocol.
Sutter et al. [55]	2016	WO/DE	Modified vaccinia Ankara virus (MVA), PmH5; MVA-MERS-S: recombinant MVA virus that expresses MERS-CoV S and N protein.	Viral Vector	Increased immunogenicity in BALB/c mice when compared to control (saline)
Zhang et al. [56]	2019	CN	MERS-CoV vaccine based on type 68 chimpanzee adenovirus and MERS-CoV S membrane protein	Viral vector	Antibodies specific titers after nasal or intramuscular injection. Neutralization of infection in cell cultures.

Abbreviations: ACE2: Angiotensin-converting enzyme-2; CRT: calreticulin; EP: European Patent Office; ID: intradermal; MVA: Modified Vaccinia Ankara (MVA) NA: Not Applicable; NSP1: Nonstructural protein 1; PFU: Plaque forming units; SC: subcutaneous; RBD: Receptor-binding domain; WO: World Intellectual Property Organization. Countries: CN: China; DE: Germany; ES: Spain; FR: France; JP: Japan; KR: Republic of Korea; TW: Taiwan; US: United States of America.

antibodies concerning the buffer solution using the same routes of administration. Moreover, the antibodies produced were able to neutralize the infection in infected cell cultures. Adenovirus vaccines that express different fragments of the SARS-CoV-1 protein S have also been studied [59,60]. A study by Shim et al. [61] sublingual immunization using this type of vaccine approach produced a mucosal and systemic immune response in an *in vivo* mouse model.

On the other hand, Sutter et al. [55] used the modified vaccinia Ankara (MVA) virus as a strategy for developing a vaccine against MERS-CoV. The MVA virus belonging to the Poxviridae family is obtained by passing chicken embryo fibroblast cultures a hundred

times. This non-replicating vector in mammalian cells, acts as one of the main recombinant vectors in preclinical and clinical research in humans for the production of new vaccines. The inventors used this viral vector platform to express the receptor-binding domain, the immunogenic fragment of the virus spike protein and the nucleocapsid protein. The intramuscular application of the vaccine in rodents using the recombinant MVA virus, having genetic sequences encoding the MERS-CoV S and N proteins, promoted neutralizing antibodies against the virus infection. It was also observed that the presence of the N gene sequence in the vaccine composition promoted better immunogenic activity compared to the use of isolated protein S [62,63].

Nucleic acid vaccine

The nucleic acid strategy comprises DNA and RNA vaccines. Four patented approaches are based on DNA vaccines and one on mRNA. Nucleic acid vaccines drive antigen synthesis in the host cell, which protects the antigen stimulates cellular and antibody immune responses [63]. They are safe and stable at room temperature due to the lack of live or attenuated pathogens.

Nucleic acid strategies also include S, M or E proteins and are always accompanied by adjuvants co-expressed or added to the vaccine formulation. These substances are necessary to enhance memory cells' immune response and production for long-term immunity [64]. All vaccine candidates elicited a cellular response in animals, and the antibodies produced by the host were able to block the virus infection in cell cultures [43–47]. Finding suitable delivery systems, and the low magnitude of immune responses, particularly in primates, are the main bottlenecks regarding nucleic acid vaccine strategies. It partly explains the lack of approved vaccines in this field.

In addition to RNA or DNA, the patented nucleic acid vaccines expressed the spike protein of SARS-CoV-1 or MERS-CoV. All were able to mediate cellular immune responses and antibody recruitment *in vivo*, and produce $CD8^+$ and $CD4^+$ T cells, thus providing an immune response to fight the virus [63–67]. DNA vaccines are capable of inducing specific immunity to cellular and humoral antigens. It is a stable platform and easy to prepare in large quantities, in addition to the security offered by the plasmid's DNA, allowing the repetitive administration of the vaccine. Among one of the proposed inventions, a DNA vaccine with antigen linked to calreticulin improved the antigen's presentation and produced specific responses of $CD8^+$ T cells [43].

In another invention, Zeng et al. [44] provide a nucleic acid vaccine by cloning the SARS-CoV S gene in the plasmid pcDNA3. The evaluation of the vaccine effect was tested in mice treated with the test vaccine (pcDNA3-SN Shuttle-SC) and negative control (empty vector pcDNA3). The animals in each group were treated with 50 μg of the vaccine in each paw, totaling 200 μg per mouse. On the seventh and 14th day the dose was increased and on the 21st day the serum was collected. At the end of the experiments carried out in triplicate, antibodies expressed by fragments of the SARS-CoV virus were detected in the serum of animals immunized with the developed vaccine [44].

Studies have already reported using a DNA vaccine based on complete spike gene approach that was capable of generating robust responses to neutralizing antibodies [39]. A phase I study developed by Modjarrad et al. of a DNA vaccine for MERS-CoV did not develop serious adverse effects attributed to the vaccine. Immune responses were induced in more than 85% of the volunteers, regardless of the doses tested after two applications and with an effect lasting one year [68].

Subunit protein vaccines

This vaccine approach used a protein from the virus or part of it to generate an immune response. The coronaviruses are single-stranded RNA viruses comprised of genome encodes, which have four structural proteins, spike (S), envelope (E), membrane (M), and nucleocapsid (N). The S protein is one of the most important in the virus structure since it is the protein used for its entry into the host cell, and can mediates viral receptor-binding and membrane infusion. Therefore, this protein may be the main factor in determining the severity of the clinical disease. During SARS-CoV and SARS-CoV-2 infection, the protein S interacted with the human angiotensin-converting enzyme 2 (ACE2) and utilizes cellular proteases cleavage, in this case the transmembrane serine protease 2 (TMPRSS2) and the endosomal cysteine proteases, cathepsin B and L. Both can enhance the virus entry using the S protein's prime, which its proteolysis become the S protein into S1 and S2, being essential to the spread of the virus. Moreover in the MERS-CoV, the same protein attacks the cellular receptor dipeptidylptyptidase 4 (DPP4) [69,70].

The S1-protein contains a receptor-binding domain (RBD), which can recognize the ACE2 and has the receptor binding motif (RBM), that makes all contacts with ACE2 and RBD. The bond between RBD and ACE2 receptor, consequently, provokes on S2 a conformational change. The S2 protein plays an important role in virus assembly and entry. Therefore, the vaccines based on S-protein can evoke the immune system to induce humoral and cellular responses and those already vaccinated from coronavirus challenges [71]. Due to this, the S protein has been one of the most promising candidates for vaccine design for coronavirus since it can induce neutralizing antibodies and develop a strong T cell response [72].

Five of the patents were based on subunit approaches and involved the proteins S, M and E associated with an adjuvant and or a delivery vector. Due to their low immunogenicity subunit vaccines frequently need an adjuvant and a delivery vector to enhance the immune response to antigens and to protect the antigen from degradation, respectively [65]. The correct selection of the delivery system and the direct adjuvant correlate with protective and long-term immune responses. Protein vaccines such as the toxoids for tetanus and pertussis and, more recently, against cervical cancer associated with HPV and B Hepatitis are licensed for human use [66].

Subunit vaccines, mostly, contain full-length S protein or portions of it, which can induce an antibody response. The S protein plays an essential role in receptor binding and membrane fusion; consequently, this can cause the decrease of the virus spread. Moreover, this type of vaccine may focus in the immune response through neutralizing epitopes. However avoiding non-neutralizing antibodies can promote antibody-dependent enhancement (ADE) disease [73,74]. After the vaccination, some full-length S proteins can induce greater infectivity and eosinophilic infiltration [75]. Moreover, the immunization of animals using parts of the S protein, such as the S1 RBD domain fused with IgG1 FC portion (RBD-FC) induced potent antibodies, which could bind with the RBD domain, neutralizing the virus completely and inhibiting the entry of SARS-CoV [76].

Thus, the selected patents that used the virus protein subunit approach also used adjuvants to improve the immune response and delivery vectors to protect the antigen. Enjuanes and Almazan used granulocyte- and granulocyte-macrophage and interleukin colony-stimulating factors as adjuvants in their vaccine containing subunits of proteins N, M, E and S, in addition to the use of plasmid as a delivery vector [48]. Burt et al. [49] developed

a vaccine containing protein S as an immunogenic component. They used baculovirus as a vector and Protollin as an adjuvant, a new type of adjuvant for intranasal vaccines [77]. Jiang et al. [50] and Tan et al. [52] used saponin and Freund's incomplete adjuvant, respectively, combined with vaccines containing protein S as the immunogenic agent that binds to a host through the receptor-binding domain (RBD). Baras et al. [51] used a plasmid to protect the protein S antigen and used lipopolysaccharides and saponin as adjuvants to stimulate an immune response. All protein subunit vaccines found were able to raise the levels of neutralizing antibodies by using the immunization pathways.

Vaccines based on MERS-CoV protein S that binds to the receptor-binding domain, in this case the DPP4, were tested in monkeys and were able to immunize these primates partially in addition to reducing pneumonia and viral load [78–80]. It was also observed that vaccines containing fragments of MERS-CoV protein S increased the production of neutralizing antibodies in *in vivo* model with mice and rabbits [81,82]. The lessons learned from MERS or SARS-CoV could help the development of an effective vaccine against SARS-CoV-2. The main strategies employed with the new coronavirus vaccine are similar to those employed previously. They are usually based on entire or fragments of spike protein expressed by viral vectors, nucleic acids or as a subunit vaccine [75]. Other lessons learned include the strong inflammatory response produced after the whole virus immunization strategies and the high level of protective antibodies elicited after immunization *via* mucosal routes [83]. Taken together, the data obtained from MERS and SARS-CoV studies are very useful for the development of a vaccine for the new coronavirus. This previous knowledge can help to enhance safety and shorten the period required to produce a licensed vaccine.

Other considerations

One year after the pandemic was announced, there are currently 181 vaccines under development in pre-clinical studies and 70 candidate vaccines in clinical evaluation. Regarding these vaccines 23 are based on protein subunits, 11 use a viral vector (non-replicating) (VVnr), 10 use DNA, 10 inactivated virus, 7 use RNA, 3 a viral vector (replicating) (VVr), 2 use virus like particle (VLP), 2 VVr and antigen presenting cell (APC), 1 live attenuated virus, and 1 VVnr and APC (updated on 19 February 2021) (Figure 6) [84–87].

In this context, regarding the use of viral RNA as a vaccine development platform, the modern biotechnology company has developed an mRNA vaccine encapsulated in lipid nanoparticles and is in phase III studies with two doses at an interval of 28 days in several countries [88]. While DNA vaccines, Inovio Pharmaceuticals is in phase II and III with a plasmid DNA vaccine with electroporation in the USA [68]. And Pfizer with BioNTech has a vaccine based on LNP-mRNA in phase III with four different formats of mRNA and target antigens [89,90]. Concerning vaccines that use protein subunits, Novavax is in phase III of a coronavirus pre-fusion protein vaccine with properties that conduct the spike protein antigen DNA to stimulate an immune response. Nucleic acid vaccines have advanced over the years through better delivery technologies, characterization methods, and biomolecules' isolation. The synergistic use of mesenchymal stem cells and RNA or DNA-based vaccines can reduce inflammation, recruit of antigen-presenting cells, and activation of B and T cells [91].

While the Wuhan Institute of biological products (Sinopharm) is in phase III trials of an inactivated virus in China, using a non-replicating viral vector, CanSino Bio is in phase III testing Adenovirus type 5 vector (Ad5-nCoV). The vaccine uses viral

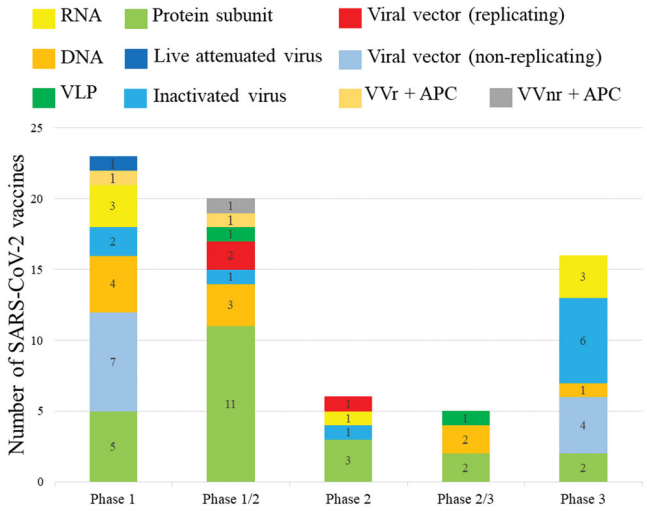

Figure 6. Number of SARS-CoV-2 vaccines by type of platform and current phase. VLP: Virus like particle; VVr (Viral vector replicating); VVnr (Viral vector non-replicating); APC (Antigen presenting cell).

vectors to carry antigens to express the SARS-CoV spike protein [87]. Still in China, in phase II, the LV-SMENP-DC vaccine, developed by Shenzhen Geno Immune Medical Institute, uses a lentiviral vector to deliver COVID-19 minigenes to modify dendritic cells and activate T cells. In addition to these trials, the vaccine developed at Oxford University in partnership with the company AstraZeneca, called ChAdOx1-S, which uses a non-replicating viral vector, is in phase III, tested on candidates in England, Brazil, and South Africa [38].

However, despite the efficacy shown by the vaccines tested in producing neutralizing antibodies and inducing cellular and humoral responses, safety is still an important requirement for any preventive strategy. The data obtained in studies developed with vaccines against coronaviruses in animals, expressed induction of non-neutralizing antibodies, worsening of viral infection, unwanted immune responses with the use of the complete portion of protein S. Thus, take preventive measures in the development of vaccines that use the full length of protein S, due to harmful immune responses derived from non-neutralizing epitopes present in the immune dominant domains. Thus, appropriate assay systems should be applied to detect potentialized virus antibodies for the new vaccine against COVID-19. However, replacement of the complete portion of protein S by the RBD portion in the development of vaccines must be taken into consideration. Additionally, the RBD portion of protein S has a lack of immune dominant domains with non-neutralizing epitopes, which makes it a safe and highly immunogenic portion. It can also induce neutralizing antibodies against mutagenic viral strains, generating a broad spectrum and protection of cross-immunity [92].

In this complete review, we describe the main patents relating to the development of vaccines for SARS-CoV and MERS, and the candidate vaccines for COVID-19 that are under clinical study worldwide. Moreover, the experience acquired with the H1N1 vaccine highlights the need for innovative development and manufacturing platforms that can be readily adapted to new pathogens [32]. Among vaccine platforms, viral vectors can provide genetic material that presents an immunogen of relevance to the host. In 2002, this approach was used for the SARS-CoV-1 outbreak, through a DNA plasmid with the glycoprotein S gene as an immunogen. In addition to the DNA platform, nanoparticles, virus-like particles (VLP) and mRNA have also been used. The use of prototypes of pathogens can promote the selection of successful

candidates in the most safely. In this type of approach, studies use the pathogen prototype, which although not itself a threat, can help to develop techniques that can be used against pathogens of interest [93]. Although scientific literature is scarce about the current development of SARS-CoV-2 vaccines, Le and colleagues and Lurie and colleagues provide an excellent source of complementary information [26,32].

It is hoped that in a few years the field of vaccine development for emerging diseases will have improved further. Better studies to evaluate the efficacy and safety of vaccines and greater investment by the public and private sector in this field. To help prevent and combat new diseases through biodefense mechanisms.

Conclusion

Respiratory diseases of viral origin are easily transmitted by infected individuals, including those that can cause severe symptoms and even death. In the case of COVID-19, despite the strong measures implemented to reduce the transmission of the virus, the number of cases and deaths still grows. Thus, obtaining a vaccine for SARS-CoV-2 is an important prophylactic strategy for disease control and prevention. Several institutions have been researching vaccine development since the SARS epidemic in 2003, followed by MERS in 2012. This research is based on viral vaccines, protein subunit vaccines, and nucleic acid vaccines that stimulate a humoral immune response, blocking the binding of the receptor-binding domain (RBD) in the S1 subunit of the SARS-CoV spike (S) protein to ACE2, or inducing an immune response from T lymphocytes. Therefore, the information presented in this review about research carried out about the SARS and MERS vaccine can reference the development of a COVID-19 vaccine. The number of new infections and deaths caused by COVID-19 is expected to decrease with vaccine administration worldwide, along with protection and isolation measures. Open science and expanding the availability of data obtained from other coronavirus outbreaks contribute to developing strategic prevention tools and to a post-pandemic life.

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We dedicate this article to all health professionals who have died or are in the front line in the against COVID-19.

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