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MINI-REVIEW

Recent advances in therapeutic modalities and vaccines to counter COVID-19/SARS-CoV-2

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ABSTRACT

The novel coronavirus disease (COVID-19) caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) has rapidly spread across the world. This resulted an alarming number of fatalities with millions of confirmed infected cases, pretending severe public health, economic, and social threats. There is no specific therapeutic drugs or licensed vaccines or treatments to fight against lethal COVID-19 infections. Given the significant threats of COVID-19, the global organizations are racing to identify epidemiological and pathogenic mechanisms of COVID-19 to find treatment regimens and effective therapeutic modalities for future prevention. Herein, we reviewed the therapeutic interventions and vaccines for COVID-19 based on the existing knowledge and understanding of similar coronaviruses, including MERS-CoV and SARS-CoV. The information constitutes a paramount intellectual basis to sustenance ongoing research for the discovery of vaccines and therapeutic agents. This review signifies the most available frontiers in the viral vaccine development approaches to counter the COVID-19/SARS-CoV-2.

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Introduction

Several coronaviruses are prevalent in humans causing only colds and slight upper respiratory infections. Nevertheless, the widespread outbreaks of coronavirus infections have become increasingly pervasive in humans. The first pandemic of the twenty-first century caused by the severe acute respiratory syndrome coronavirus (SARS-CoV) emerged in 2002–2003. After a decade, the Middle East respiratory syndrome coronavirus (MERS-CoV) appeared, and infections continue to expand in the Middle East. Now, after seven years of MERS-CoV, another coronavirus, SARS-CoV-2 with more devastating effects has again emerged,[1,2] and the associated disease COVID-19 has disseminated to over 200 different countries of the world. Across virtually all counties worldwide, millions of people have been infected, and hundreds of thousands have died.[3] Additionally, the lives of millions of people have been substantially affected because of quarantines and mandatory isolations. The ripple influence of this pandemic has brought potential challenges to worldwide healthcare systems and might trigger a "far-reaching" global economic crisis if the virus spread is not strictly curbed.[4,5] Given the sequence analysis, the genome of SARS-CoV-2 shows 80% and 54% close relation to SARS-CoV and MERS-CoV, respectively.[6] Until now, there is a very limited arsenal to fight against lethal infections with no officially registered vaccines or treatments to combat any of these deadly CoV-induced syndromes, including SARS-CoV-2. SARS-CoV-2 binds to the similar host-cell receptor (angiotensin-converting enzyme 2) and might exhibit common pathogenic mechanisms and limited cross-neutralizing antibodies.[6,7] This acquaintance along with improved knowledge of coronavirus reproduction approaches and improvements in vaccine technology since SARS-CoV advocates that vaccine for COVID-19 might be coming more promptly than earlier but regrettably may not be available to curtail the recent outbreak.

SARS-CoV-2 possesses a large positive-sense single-stranded RNA genome (29.88 Kb) consisting of about 12 open reading frames. Similar to all other CoV, SARS-CoV-2 also encodes genes responsible for the production of four major structural proteins, explicitly the nucleocapsid related to the RNA genome, and a set of three other membrane proteins. These proteins include the integral membrane glycoprotein, large spike glycoprotein, and the envelope protein. The large spike protein comprises the receptor-binding domain and plays a significant role in the membrane fusion and viral attachment to host cells. It also helps to induce viral neutralizing antibodies, which might inhibit interactions with the host receptors.[6] It is the major focus for vaccine research and development. The integral membrane glycoprotein can also trigger neutralizing antibodies, and the nucleocapsid protein comprises T-cell epitopes also indicates an important vaccine target for SARS-CoV.[8-10]

Anti-viral therapies

Interferon-α (IFN-α) is a member of type I interferons family that provides one of the frontlines of host resistance against viral invade and infectivity. IFN-α inhibits virus infection by direct interference with the replication cycle and boosting up innate and acquired immune responses to infection. In-vitro trials-based studies revealed the effective ability of IFN-α to

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suppress the reproduction of SARS-CoV.[11,12] IFN-α-vaccinated Cynomolgus monkeys have been found to be less vulnerable and protected from infectious diseases caused by SARS-CoV.[13] Furthermore, the therapeutic benefits of synthetic recombinant IFN-α have been demonstrated for patients infected with SARS-CoV in a pilot-scale investigation.[14] Therefore, IFN-α might be advocated as a candidate medication for the therapeutic treatment of SARS-CoV-2.

Lopinavir-ritonavir was recognized as a first protease-inhibiting agent,[15,16] which can attach to the endopeptidase C30 of SARS-CoV-2 protease,[17] and thus exerting potential anti-viral activities by impeding the formation of key proteins of the SARS-CoV-2. Evidence suggests that lopinavir-ritonavir treatment either in individual or amalgamation with other anti-viral therapies has improved the outcome of MERS-CoV or SARS-CoV infected patients by ameliorating acute respiratory distress syndrome (ARDS).[18-20] A comparative illustration of healthy and infected alveolus due to ARDS is shown in Figure 1. Considering the similar nature SARS-CoV-2 with SARS-CoV or MERS-CoV, lopinavir-ritonavir could exhibit a beneficial impact to encounter COVID-19 infectious disease. However, additional investigations are required to elucidate this opportunity.

Arbidol is utilized as a unique anti-viral candidate that has revealed a robust in-vitro influence in suppressing the replication of the SARS-CoV.[21] Case reports evidenced the anti-viral potency of sole arbidol or in blend with other drugs when used for dealing with pneumonia caused by SARS-CoV-2.[22-26] Presently, many clinical control trials are being executed to examine the effectiveness of arbidol against SARS-CoV-2. Remdesivir is a nucleoside analogue GS-5734 with broad-spectrum activity to suppress SARS-CoV and MERS-CoV *in vivo*.[27,28] It strappingly prevented SARS-CoV -2 infection in a recent *in vitro* investigation at low-micro molar concentration accompanied by a high selectivity index.[22] Moreover, the first patient of COVID-19 in the United States was successfully treated by the intravenous administration of remdesivir.[29] Though remdesivir exhibits many merits for curing SARS-CoV-2 infection and pneumonia, randomized controlled trials (RCTs) are still necessitated to prove its effectiveness and safety status.

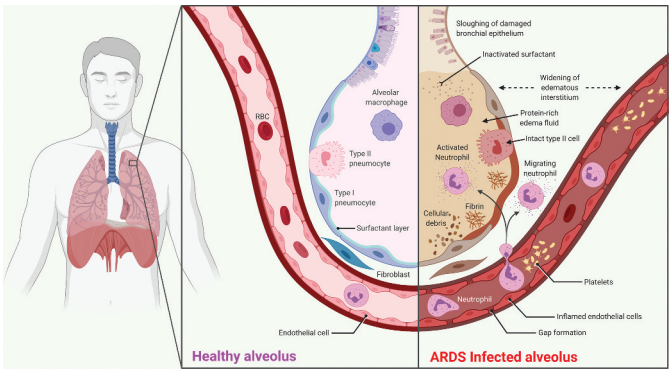

Figure 1. A comparative illustration of healthy and infected alveolus due to Acute Respiratory Distress Syndrome (ARDS). The Figure was created with "BioRender.com" template and exported under the terms of premium subscription.

In conclusion, all the aforementioned anti-viral drugs might be prospective choices to cure COVID-19, but a few important points should be noted as follows.[30,31]

1) The potential drug-drug interactive effect of these anti-viral preparations with other remedial drugs should be deliberated.

2) The possible antagonistic events due to lopinavir/ritonavir, such as vomiting, nausea, diarrhea, and liver impairment should also be noticed.

3) Taking three or more anti-viral medications is not recommended at the same time, and the consumption of these therapeutic drugs should be discontinued in case of drug-related intolerable adverse effects.

4) Additional efficacy assessment of currently used anti-viral drugs is required for clinical applications.

Therapeutic treatments for COVID-19 targeting protease inhibitors

Chymotrypsin-like inhibitors

Chymotrypsin-like (3C-like) and papain-like protease (PLP) are two proteins encoded by a coronavirus. In addition to exhibit a key role in the replication of coronavirus, both of these proteins also possess the function to inhibit innate immune responses of the host. Therefore, these proteins could be attractive anti-viral drug targets to deal with the coronavirus.[32] Cinanserin is a well-known serotonin antagonist. Due to the suppression of 3-chymotrypsin-like protease, cinanserin was considered as a potential and timely candidate for inhibiting the reproduction of SARS-CoV.[33] Since 3-chymotrypsin-like protease has also been determined in COVID-19,[23,24] thus, cinanserin can be a suitable therapeutic option for suppressing the infection caused by SARS-CoV-2. Flavonoids are an imperative group of naturally occurring products, including many subgroups such as flavones, chalcones, flavonols, and isoflavones.[34] Apart from well-known antioxidant properties, these compounds also possess promising anti-viral effects. Jo et al.[35] revealed that the anti-corona viral properties of bioactive flavonoids, including rhoifolin, Herbacetin, and pectolinarin are attributed to the hampering of 3C-like protease. Some flavonoid compounds particularly isobavachalcone, helichrysetin, Herbacetin, and quercetin 3-β-d-glucoside have also shown ability to inhibit the enzyme activity of MERS-CoV. Similarly, bioflavonoids from *Torreya nucifera* caused the blockage of SARS-CoV replication.[36]

Papain-like protease inhibitors

Papain-like protease encoded by human coronavirus has a unique and novel deubiquitinating activity. It is a promising interferon antagonist and inhibits the inherent immunological response of the host against the virus. Diarylheptanoids is a bioactive compound that can be obtained from the stem bark of Japanese alder plant. It exhibited the inhibiting ability of the papain-like protease of SARS-CoV.[32] Hence, cinanserin in combination with other natural biologically active substances such as flavonoids or diarylheptanoids may be picked as alternative therapeutic options in combating COVID-19-induced infections via targeting coronavirus proteases.[24]

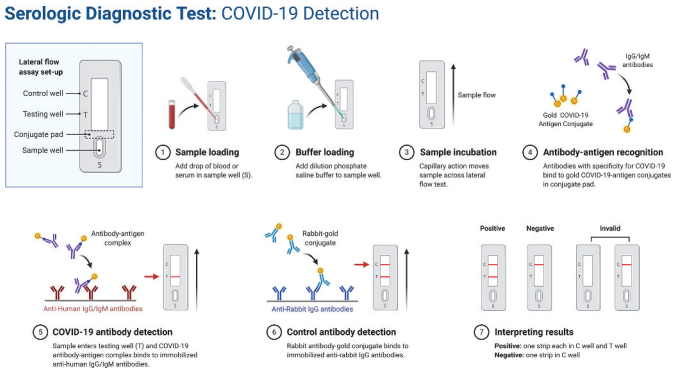

Figure 2. A stepwise illustration of COVID-19 serologic diagnostic test through antibody detection. The Figure was created with "BioRender.com" template and exported under the terms of premium subscription.

Spike (S) protein-angiotensin converting enzyme-2 (ACE2) blockers

Angiotensin-converting enzyme-2 (ACE2), a type I essential membrane protein, catalyzes the role of a carboxypeptidase. Reports have revealed that it serves as a functional receptor of SARS-CoV coronavirus and facilitates the entry of the virus into target cells via binding with spike (S) glycoprotein.[37] As a type I surface protein, S protein is associated with the attachment to cell receptors and intervenes in the fusion of host and viral membranes.[38]

Human monoclonal antibody

Monoclonal antibodies (mAbs) characterize a major class of bio-therapeutic products as passive immunotherapies in combating viral infections. Figure 2 shows a stepwise illustration of the COVID-19 serologic diagnostic test through antibody detection. The therapeutic uses of mAbs have been widely accepted and acquired remarkable achievements in the treatment of numerous chronic and lethal viral diseases.[39] From the two nonimmune human antibody libraries, Sui et al.[40] found one recombinant human mAb that efficiently neutralized the SARS-CoV and inhibited the formation of syncytia between the S-protein and SARS-CoV receptor ACE2.

Chloroquine, emodin and promazine

Chloroquine is a 9-aminoquinoline that has recently been described as a potent drug with significant anti-viral activity.[41] Chloroquine has been known to be a potent inhibitor of SARS-CoV-induced infectious disease by disrupting ACE2, which is important binding regions on cell surfaces for S protein of SARS-CoV.[42] Wang et al.[43] demonstrated the effective suppression activity of chloroquine for the newly emerged novel CoVID-19 *in vitro*. Out of five different FDA-registered broad-spectrum drugs and two anti-viral agents tested against a new clinical isolate of SARS-CoV-2, chloroquine was observed to be extremely efficacious in preventing and controlling SARS-CoV-2 infection.[43] It is a cheap drug, which has been utilized for more than seven decades, and hence it could be a potentially applicable choice against COVID-19. Further, a safe track promotes its assessment in human patients infected with SARS-CoV-2 disease (COVID-19).[44]

Emodin is a natural anthraquinone compound that can act as a virucidal agent,[45] and considerably interfere with the binding interactions between the ACE2 and SARS-CoV S-protein. Thus, it is thought that emodin could eliminate SARS-CoV triggered disease conditions by acting as a competitor for the same binding region of S-protein with ACE2.[46] Promazine is an anti-psychotic medication that shares a structural similarity with emodin. It has been shown a pronounced suppressing activity to inhibit the growth of SARS-CoV.[47] In contrast to emodin, promazine is a more potent inhibitor of the attachment of spikes glycoproteins to ACE2. The investigation portrays that promazine and emodin might inhibit SARS-CoV disease by interfering with the binding of ACE2 and S protein.[46] Consequently, the mAbs of promazine and emodin can be employed as alternatives to treat COVID-19.

Candidate vaccines for SARS-CoV, MERS-CoV, and COVID-19

It is extremely critical to discover effective and safe vaccines to counter COVID-19 pandemic, limit its rapid dissemination, and eventually circumvent its future reappearance. Table 1 summarizes an overview of vaccine production platforms and vaccine candidates for various Coronaviruses.[31,48-55] To date, no vaccines have been approved in humans to combat coronavirus-induced respiratory infections. Only IBV vaccines have been registered in animals for the management of upper respiratory-tract infections of CoV in chickens.[56,57] Since the COVID-19 shares substantial sequence similarity with the earlier two devastating coronaviruses (MERS-CoV and SARS-CoV), the vaccinations developed for MERS-CoV and SARS-CoV would greatly expedite the development of anti-COVID -19 vaccines. Important criteria to be considered for all vaccines include the protective efficacy, safety, and duration of vaccine-acquired immunity, but the high manufacturing capacity and rapid development is also indispensable for vaccines in the face of emerging pandemics.[10] Even though attenuated and traditional inactive vaccines are being appraised, high-titer preparation of these vaccines in cell culture under the conditions of biosafety level-3 is a great challenge.

The newer anti-viral vaccines commonly fall into following types such as inactive or live-attenuated viruses, viral vectors, virus-like particle (VLP), DNA-based, mRNA-based, and protein-based vaccines.[58] It is documented that viral S protein subunit vaccines generate elevated titers of neutralizing antibodies and provide more protection compared to full-length S protein, DNA-based S protein and live-attenuated SARS-CoV vaccines.[59] It is not surprising that approximately half of the patented reports have focused on the viral S protein subunit vaccine and those explicitly directing the RBD of the S1-subunit. Notably, S gene/protein is considered as the preferred targeting site in the development of the SARS-CoV/MERS-CoV vaccine, and thus, this approach can also be used in designing vaccines for SARS-CoV-2.

Table 1. Overview of vaccine production platforms and vaccine candidates for various Coronaviruses.

Vaccine platform	Vaccine candidate	Target antigen	Animal model	PMID	PMCID	References
Parainfluenza virus 5 (PIV5)-based vaccine	PIV5/MERS-S	S protein	Yes – Specific-pathogen-free 6-week-old C57BL/6 and BALB/c mice	32265331	PMC7157776	Li et al.[31]
Vector vaccine	Modified vaccinia virus Ankara (MVA)-based vaccine	S protein	No	32325037	PMC7172913	Koch et al.[48]
Recombinant	VSVΔG-MERS	S protein	Yes – eight 2-year old male rhesus monkeys	29246504	PMC7113862	Liu et al.[49]
DNA vaccine, Recombinant	pcDNA3.1-S	S protein	Six-to eight-week-old specific pathogen-free female BALB/c mice	28314561	PMC5411280	Chi et al.[50]
DNA vaccine, Recombinant	pcDNA3.1-SΔCD	S protein	Six-to eight-week-old specific pathogen-free female BALB/c mice	28314561	PMC5411280	Chi et al.[50]
DNA vaccine, Recombinant	pcDNA3.1-S1	S protein	Six-to eight-week-old specific pathogen-free female BALB/c mice	28314561	PMC5411280	Chi et al.[50]
DNA vaccine live-attenuated *S. typhimurium*	pcDNA-N	N protein	Yes – Eight-week-old female BALB/c mice	19186202	PMC7115532	Hu et al.[51]
DNA vaccine	pcDNA-S	S protein	Yes – Six- to 8-week-old female BALB/c mice	17494640	PMC1951058	Hu et al.[52]
DNA vaccine	pcDNA-IL-2	S protein	Yes – Six- to 8-week-old female BALB/c mice	17494640	PMC1951058	Hu et al.[52]
DNA vaccine	pcDNA-S + pcDNA-IL-2	S protein	Yes – Six- to 8-week-old female BALB/c mice	17494640	PMC1951058	Hu et al.[52]
DNA vaccine	pcDNA3.1	S protein	Yes – Six- to 8-week-old female BALB/c mice	17494640	PMC1951058	Hu et al.[52]
DNA vaccine	PBS	S protein	Yes – Six- to 8-week-old female BALB/c mice	17494640	PMC1951058	Hu et al.[52]
DNA vaccine	CS022	S protein	Yes – Six- to 8-week-old female BALB/c mice	17494640	PMC1951058	Hu et al.[52]
DNA vaccine	N protein DNA vaccine	N protein	Yes – Female Balb/c mice	16423399	PMC7112551	Shi et al.[53]
DNA vaccine	M protein DNA vaccine	M protein	Yes – Female Balb/c mice	16423399	PMC7112551	Shi et al.[53]
DNA vaccine	N + M protein DNA vaccine	N, M proteins	Yes – Female Balb/c mice	16423399	PMC7112551	Shi et al.[53]
DNA vaccine	CTLA4-S DNA vaccine	S protein	Yes – Male Balb/c (H-2^d) mice	15993989	PMC7115571	Woo et al.[54]
DNA vaccine	*Salmonella*-CTLA4-S DNA vaccine	S protein	Yes – Male Balb/c (H-2^d) mice	15993989	PMC7115571	Woo et al.[54]
DNA vaccine	*Salmonella*-tPA-S DNA vaccine	S protein	Yes – Male Balb/c (H-2^d) mice	15993989	PMC7115571	Woo et al.[54]
Recombinant	Recombinant spike polypeptide vaccine	S protein	Yes – Male Balb/c (H-2^d) mice	15993989	PMC7115571	Woo et al.[54]
DNA vaccine	tPA-S DNA vaccine	S protein	Yes – Male Balb/c (H-2^d) mice	15993989	PMC7115571	Woo et al.[54]
DNA vaccine	N protein DNA vaccine	N protein	Yes – Six-week-old female BALB/c mice	15582659	PMC7111813	Zhao et al.[55]

Attenuated virus vaccines

Live-attenuated CoV vaccines are produced by reverse genetics from infectious virus clones by deleting numerous key pathogenicity factors to avoid reversion. These vaccines have considered the immunologically most robust that induce systemic, mucosal, humoral, and cellular-mediated immune responses and broad cross-protective immunity.[2,8,9,60] The best approach to produce an attenuated vaccine for COVID-19 would be first constructing a temperature-sensitive virus mutant with replication constrained to the upper respiratory tract, and then generate additionally-conserved attenuated mutations by applying reverse genetics. In blending with a parental heterologous RNA genome-associated nucleocapsid and large spike glycoprotein booster vaccine, such kind of attenuated vaccine can profoundly provoke cross-protection against different strains like bat SARS-CoV-like strains 1. The live-attenuated coronavirus or Toro virus vaccines are disclosed in patent application US20060039926.[61] Insertion of a mutation (Y6398H) into the Orf1a/b polyprotein (p59/nsp14/ExoN) abolished the pathogenicity of mouse coronavirus (MHV-A59). The resultant attenuated MHV virus showed weaned reproduction in the tested mice after intracerebral vaccination.

Virus-like particle or subunits vaccines

Viral subunits or virus-like particles vaccines provide universal and useful vaccine platforms against a variety of emerging viruses.[10] These vaccines closely imitate attenuated vaccines by destroying host cells or the induction of endogenous antigens and thus results in the generation of both T-cell and antibody-mediated immune responses.[8-10,60] The candidate vaccines under research and development for COVID-19 include the large spike protein or receptor-binding domain subunit vaccines and replicating or non-replicating viral vector-based vaccines with the expression of large spike protein or receptor-binding domain.[62] Patent application WO2015042373 revealed an immunogenic preparation comprising MERS-CoV nanoparticles virus-like particle vaccines that contained at least one trimer of an S protein secreted by overexpressed baculovirus in Sf9 cells.[63] The following administration together with proprietary adjuvant Matrix, this VLP composition provoked a counteracting antibody response in transgenic cattle and mice. Furthermore, the injection of sera preparations from vaccinated cattle (SAB-300 or 301) into Ad5-hDPP4 transduced BALB/c mice were capable of protecting these mice from MERS-CoV attack by using only one prophylactic inoculation. On February 26, 2020, Novavax has announced starting animal testing on COVID-19 vaccine candidates because of their earlier experiences with SARS-CoV and MERS-CoV.[64] They developed COVID-19 candidate vaccines targeting the S protein of SARS-CoV-2 utilizing engineered nanoparticle vaccine technology accompanied by proprietary adjuvant Matrix-M.

DNA-based vaccines

Although DNA-based vaccines are recognized as stable, safe, and produced at a fast rate, their protective efficiency and immunogenicity in humans have not yet confirmed. DNA vector vaccines for MERS-CoV and SARS-CoV have often shown better effectiveness heterologous prime-boost (inactivated virus particles, S/S1 proteins, and recombinant viral vectors) regimens.[8,9,60] However, the administration of DNA vaccine by an electroporation technique and its possible host genome integration and persistence are the issues to be solved.[10] The compositions and approaches for boosting up immunological responses, predominantly antigen-specific $CD8^+$ T-cell-assisted responses against SARS-CoV infection are documented in patent application WO2005081716.[65] Immune responses, particularly cytotoxic T cell-mediated immune responses, are enhanced in vivo by chimeric nucleic acids encoding endoplasmic reticulum chaperone polypeptides, which are connected to at least one antigenic protein from SARS-CoV. The effective delivery of DNA-based gold particles by using a gene gun method, immunization of mice against a calreticulin–nucleocapsid fusion protein showed powerful T cell-mediated immune and nucleocapsid-specific humoral responses. The titer of a vaccinia vector with the expression of N protein of the SARS-CoV was significantly reduced in vaccinated animals. Immunogens containing consensus proteins originated from the spike protein of MERS-CoV for utilization in MERS-CoV specified DNA-based vaccines are patented in application WO2015081155.[66] The consensus spike protein considerably enhanced both cellular and humoral immune responses, such as high-tittered neutralizing antibodies and specific immunoglobulins. Induced cell-mediated immunity involved amplified responses of CD3 + CD4+ and CD3+ CD8 + T cells, which result in the production of TNF-α, IFN-γ, IL-2, or both TNF-α and IFN-γ.

mRNA-based vaccines

mRNA vaccines are used as prototypes for the expression and production of endogenous proteins in the vaccinated subject. The application of lipid nanoparticles can enhance the delivery efficacy of the mRNA vaccines for intradermal or intramuscular administration.[10] mRNA-based prophylactic vaccines offer the potential advantage of the anticipated development of a portable mRNA 'printing' facility for the production of huge amounts of mRNA.[2] In addition, they also provide benefits such as the capability to inducing a more powerful immunological response and the aptitude of combining several mRNAs into a single vaccine. The patent application (WO2017070626)[67] by Moderna demonstrated mRNA vaccines consists of mRNAs encoding antigenic viral full-length S, S1, or S2 proteins from MERS-CoV and SARS-CoV viruses, and prepared in cationic lipid nanoparticles. It was found that mice inoculated with mRNA encoding coronavirus full-length S protein resulted in much greater titers of neutralizing antibody than that to mRNA encoding the S protein S2 subunit. On February 24, 2020, Moderna declared the release of the first batch of mRNA-1273 against COVID-19 for utilization in humans, formulated according to the strategies and methods delineated in their earlier patents.[68]

Protein-based vaccines

A vaccine, comprising of an S protein immunogen and oil-in-water emulsion, can provoke a potential protecting immunological

response against SARS-CoV is disclosed in patent application WO2010063685 by GlaxoSmithKline.[69] Combination of an emulsion adjuvant with engineered ectodomain immunogen produced high-tittered anti-SARS-CoV IgG2a or IgG2b as well as specific neutralizing antibody responses in studied animal models. Patent application US20060002 947 unveils the formulation methodology for Ii-Key/MHC II SARS-CoV hybrids comprising of three components namely an invariant (Ii) chain peptide for antigen presentation improving activity, a chemical structure connecting the Ii with antigen epitope, and an antigenic epitope, which binds to an MHC class II molecule.[70] Recently, Generex Company contracted an agreement with a Chinese consortium involving China Technology Exchange, Biology Institute of Shandong Academy of Sciences, Beijing Zhonghua Investment Fund Management, and Sinotek-Advocates International Industry Development to design and develop a vaccine for COVID-19. It is important to note that Generex will exploit its patented Ii-Key immune system activation technology to manufacture COVID-19 viral peptide for human experimental trials.[71]

COVID-19 vaccine strategies depending on SARS-CoV-2 pathogenesis in the host

A clearer apprehension of SARS-CoV-2 pathophysiology in humans, immunological protection correlation, and the time duration of naturally acquired immunity would benefit the development of COVID-19 vaccination approaches to limit the dissemination of the virus. Comprehensive knowledge of the SARS-CoV-2 pathogenesis, including the infection of target organs and the route and mechanism of virus propagation to these vital organs, will aid in developing vaccines for the protection of target organs. For example, if the SARS-CoV-2 causes pneumonia via upper respiratory tract infection, the use of vaccine preparations containing live replication-competent vectors or weekend viruses that are able to induce local mucosal immune response can protect both the upper as well as lower respiratory tract infections and causes a significant reduction in nasal shedding. On the other hand, if the infection occurs in the lungs or other organs through viremia, then parenteral (IM) vaccines, which provoke adequate virus-neutralizing antibodies in serum to prevent viremia. Furthermore, people who have recovered from COVID-19 infection but are again primed to the virus-like seasonal influenza, the administration of a parental vaccine (i.e., subunit S or RBD protein) may provide an effective remedy as to yearly booster vaccination.[2] These booster vaccines would augment memory T- and B-cell mediated immunity responses, and may aid in preventing viral reinfections. In some COVID-19 patients, diarrhea and fecal shedding were observed,[72] and these conditions can be effectively overcome by the use of oronasal vaccines. Therefore, COVID-19 vaccination is likely to be used in three types of populations (1) naïve (susceptible) individuals with no immunity level (2) recovered individuals with different immunity levels and people having preexisting resistance to MERS-CoV or SARS-CoV. As a result, the immune reactions, defensive ability, and adverse response of candidate vaccines may differ among these populations. It will be meaningful to assess preexisting immunity levels for validating the safety and efficacy of the vaccine in each population and various range groups within these populations.[2]

Concluding remarks, current challenges and directions

In the scenario of this deadly pandemic, it is very critical to rapidly develop, produce, and deploy first-generation vaccines. A combination of synthetic nucleic acid (DNA or mRNA)-based vaccination with S (and possibly N) glycoprotein booster are leading candidate vaccines taken into consideration the aforementioned criteria. Issuance of conditional licensures is an approach that can be employed to expedite the utilization of veterinary vaccines during the COVID-19 epidemics based on human safety and protection levels to decrease mortalities in the highest-risk populations (especially elder, healthcare workers and patients with multiple comorbidities). Furthermore, more efficacious and potent second-generation vaccines should be prepared in parallel to greatly reduce disease, prevent disability, number of deaths, and block shedding. Since the virulence mechanism of SARS-CoV-2 in humans remains uncertain, therefore, vaccine strategies should also need to be changed if the virus attacks both the enteric and respiratory tracts and is also shedding in the stool of infected people. Parenteral S vaccine booster and oronasal vaccine prime might be the optimal candidates for preventing both respiratory and intestinal infections, as well as blocking nasal and fecal shedding as have been deployed for some animal CoV. It is highly likely to the spillover of SARS-CoV or SARS-CoV-2-like CoVs from potential animal reservoirs in the future. These facts warrant developing newer approaches for vaccine generation with the potential to provoke wider heterologous and cross-protection immunity against coronavirus within each betaCoV family. For this, the focus should be devoted to targeting additional proteins (N, S2, etc.) and conserved internal virus epitopes that have shown the capacity to induce broad-spectrum cross-protective and cross-reactive immunity.

Currently, the lack of approved vaccines to inducing active immunity calls for the prompt development and deployment of passive immunization approaches for prophylactic and therapeutic treatment of patients. Convalescent plasma treatment using plasma-containing antibodies from recovered patients would be the empirical and timeliest therapy during the outbreak of COVID-19. Reports have shown a shorter hospital stay and reduced rate of mortality in convalescent plasma-treated SARS-CoV patients compared with the untreated individuals.[73,74] If found effective, then he plasma banks with volunteer blood donation by a large number of recovered COVID-19 people should be established promptly.

SARS-CoV-2 – most concerned question[7]

- How much do we know about the human-to-human transmission of SARS-CoV-2?
- Does the SARS-CoV-2 appear to be significantly contagious in a human-to-human context?
- What makes SARS-CoV-2 strain significantly concerning, regardless of its resembling symptoms from the common cold to as severe as SARS-CoV?
- How concerned should we be about the SARS-CoV-2 origin, although there are more confirmed cases around the globe and the infected cases are keep growing?

- What are the main symptoms of SARS-CoV-2, and how are they distinguishable compared to the typical common cold and seasonal flu?
- What do people need to know to take preventive measures to protect themselves and avoid getting sick?
- Are the currently available viral vaccine development approaches good enough to tackle SARS-CoV-2, effectively?

Author contributions

Both authors equally contributed to the conception, design, analysis, and interpretation of data, checking and approving the final version of the manuscript, and agree to be accountable for its contents.

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References

1. Iqbal HMN, Romero-Castillo KD, Bilal M, Parra-Saldivar R. The emergence of novel-coronavirus and its replication cycle-an overview. J Pure Appl Microbiol. 2020;14(1):13–16. Article: 6146. doi:10.22207/JPAM.14.1.03.
2. Saif LJ. Vaccines for COVID-19: perspectives, prospects, and challenges based on candidate SARS, MERS, and animal coronavirus vaccines. EuroMed J. 2020. doi:10.33590/emj/200324.
3. WHO, Situation report – 92. [accessed 2020 April 22]. https://www.who.int/docs/default-source/coronaviruse/situation-reports/20200421-sitrep-92-covid-19.pdf?sfvrsn=38e6b06d_4.
4. Gorbalenya AE, Baker SC, Baric R, Groot RJD, Drosten C, Gulyaeva AA, Penzar D. Severe acute respiratory syndrome-related coronavirus: the species and its viruses–a statement of the coronavirus study group. BioRxiv. 2020. doi:10.1101/2020.02.07.937862v1.
5. Kupferschmidt K, Cohen J. Will novel virus go pandemic or be contained? Science. 2020;367(6478):610–11. doi:10.1126/science.367.6478.610.
6. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Chen HD. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature. 2020;579(7798):270–73. doi:10.1038/s41586-020-2012-7.
7. Bilal M, Nazir MS, Parra-Saldivar R, Iqbal HMN. 2019-nCoV /COVID-19 - approaches to viral vaccine development and preventive measures. J Pure Appl Microbiol. 2020;14(1):25–29. Article: 6168. doi:10.22207/JPAM.14.1.05.
8. Enjuanes L, DeDiego ML, Álvarez E, Deming D, Sheahan T, Baric R. Vaccines to prevent severe acute respiratory syndrome coronavirus-induced disease. Virus Res. 2008;133(1):45–62. doi:10.1016/j.virusres.2007.01.021.
9. Roper RL, Rehm KE. SARS vaccines: where are we? Expert Rev Vaccines. 2009;8(7):887–98. doi:10.1586/erv.09.43.
10. Rauch S, Jasny E, Schmidt KE, Petsch B. New vaccine technologies to combat outbreak situations. Front Immunol. 2018;9:1963. doi:10.3389/fimmu.2018.01963.
11. Ströher U, DiCaro A, Li Y, Strong JE, Aoki F, Plummer F, Feldmann H. Severe acute respiratory syndrome-related coronavirus is inhibited by interferon-α. J Infect Dis. 2004;189(7):1164–67. doi:10.1086/382597.
12. Zorzitto J, Galligan CL, Ueng JJ, Fish EN. Characterization of the antiviral effects of interferon-α against a SARS-like coronoavirus infection in vitro. Cell Res. 2006;16(2):220–29. doi:10.1038/sj.cr.7310030.
13. Haagmans BL, Kuiken T, Martina BE, Fouchier RA, Rimmelzwaan GF, Van Amerongen G, Tashiro M. Pegylated interferon-α protects type 1 pneumocytes against SARS coronavirus infection in macaques. Nat Med. 2004;10(3):290–93. doi:10.1038/nm1001.
14. Loutfy MR, Blatt LM, Siminovitch KA, Ward S, Wolff B, Lho H, Kain KC. Interferon alfacon-1 plus corticosteroids in severe acute respiratory syndrome: a preliminary study. JAMA. 2003;290(24):3222–28. doi:10.1001/jama.290.24.3222.
15. Walmsley S, Bernstein B, King M, Arribas J, Beall G, Ruane P, Brun S. Lopinavir–ritonavir versus nelfinavir for the initial treatment of HIV infection. New Engl J Med. 2002;346(26):2039–46. doi:10.1056/NEJMoa012354.
16. Pulido F, Arribas JR, Delgado R, Cabrero E, González-García J, Pérez-Elias MJ, Rubio R. Lopinavir-ritonavir monotherapy versus lopinavir-ritonavir and two nucleosides for maintenance therapy of HIV. AIDS. 2008;22(2):F1–F9. doi:10.1097/QAD.0b013e3282f4243b.
17. Lin S, Shen R, Guo X. Molecular modeling evaluation of the binding abilities of ritonavir and lopinavir to wuhan pneumonia coronavirus proteases. BioRxiv. 2020. doi:10.1101/2020.01.31.929695.
18. Chu CM, Cheng VCC, Hung IFN, Wong MML, Chan KH, Chan KS, Peiris JSM. Role of lopinavir/ritonavir in the treatment of SARS: initial virological and clinical findings. Thorax. 2004;59(3):252–56. doi:10.1136/thorax.2003.012658.
19. Zumla A, Chan JF, Azhar EI, Hui DS, Yuen KY. Coronaviruses—drug discovery and therapeutic options. Nat Rev Drug Discov. 2016;15(5):327. doi:10.1038/nrd.2015.37.
20. Sheahan TP, Sims AC, Leist SR, Schäfer A, Won J, Brown AJ, Spahn JE. Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nat Commun. 2020;11(1):1–14. doi:10.1038/s41467-019-13940-6.
21. Khamitov RA, Loginova S, Shchukina VN, Borisevich SV, Maksimov VA, Shuster AM. Anti-viral activity of arbidol and its derivatives against the pathogen of severe acute respiratory syndrome in the cell cultures. Voprosy Virusol. 2008;53:9–13.
22. Wang Z, Chen X, Lu Y, Chen F, Zhang W. Clinical characteristics and therapeutic procedure for four cases with 2019 novel coronavirus pneumonia receiving combined Chinese and Western medicine treatment. Biosci Trend. 2020;14:64–68. doi:10.5582/bst.2020.01030.
23. Zhang J, Zhou L, Yang Y, Peng W, Wang W, Chen X. Therapeutic and triage strategies for 2019 novel coronavirus disease in fever clinics. Lancet Respir Med. 2020;8(3):e11–e12. doi:10.1016/S2213-2600(20)30071-0.
24. Zhang L, Liu Y. Potential interventions for novel coronavirus in China: a systemic review. J Med Virol. 2020;92:479–90. doi:10.1002/jmv.25707.
25. Zhang N, Wang L, Deng X, Liang R, Su M, He C, Du L. Recent advances in the detection of respiratory virus infection in humans. J Med Virol. 2020;92(4):408–17. doi:10.1002/jmv.25674.
26. Xu XW, Wu XX, Jiang XG, Xu KJ, Ying LJ, Ma CL, Sheng JF. Clinical findings in a group of patients infected with the 2019 novel coronavirus (SARS-Cov-2) outside of Wuhan, China: retrospective case series. Bmj. 2020;368:m606. doi:10.1136/bmj.m606.
27. Agostini ML, Andres EL, Sims AC, Graham RL, Sheahan TP, Lu X, Ray AS. Coronavirus susceptibility to the anti-viral remdesivir (GS-5734) is mediated by the viral polymerase and the proofreading exoribonuclease. MBio. 2018;9(2):e00221–18. doi:10.1128/mBio.00221-18.
28. de Wit E, Feldmann F, Cronin J, Jordan R, Okumura A, Thomas T, Feldmann H. Prophylactic and therapeutic remdesivir (GS-5734) treatment in the rhesus macaque model of MERS-CoV infection.

Proc Natl Acad Sci. 2020;117(12):6771–76. doi:10.1073/pnas.1922083117.
29. Holshue ML, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H, Diaz G. First case of 2019 novel coronavirus in the United States. New Engl J Med. 2020;382:929–36. doi:10.1056/NEJMoa2001191.
30. Li H, Liu SM, Yu XH, Tang SL, Tang CK. Coronavirus disease 2019 (COVID-19): current status and future perspective. Int J Antimicrob Agents. 2020;55:105951. doi:10.1016/j.ijantimicag.2020.105951.
31. Li K, Li Z, Wohlford-Lenane C, Meyerholz DK, Channappanavar R, An D, He B. Single-dose, intranasal immunization with recombinant parainfluenza virus 5 expressing middle east respiratory syndrome coronavirus (MERS-CoV) spike protein protects mice from fatal MERS-CoV infection. mBio. 2020;11(2):e00554–20. doi:10.1128/mBio.00554-20.
32. Park JY, Jeong HJ, Kim JH, Kim YM, Park SJ, Kim D, Ryu YB. Diarylheptanoids from Alnus japonica inhibit papain-like protease of severe acute respiratory syndrome coronavirus. Biol Pharm Bull. 2012;35:2036–42. doi:10.1248/bpb.b12-00623.
33. Chen L, Gui C, Luo X, Yang Q, Günther S, Scandella E, Chen J. Cinanserin is an inhibitor of the 3C-like proteinase of severe acute respiratory syndrome coronavirus and strongly reduces virus replication in vitro. J Virol. 2005;79(11):7095–103. doi:10.1128/JVI.79.11.7095-7103.2005.
34. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. J Nutr Sci. 2016;5:e47. doi:10.1017/jns.2016.41.
35. Jo S, Kim S, Shin DH, Kim MS. Inhibition of SARS-CoV 3CL protease by flavonoids. J Enzyme Inhib Med Chem. 2020;35(1):145–51. doi:10.1080/14756366.2019.1690480.
36. Ryu YB, Jeong HJ, Kim JH, Kim YM, Park JY, Kim D, Rho MC. Biflavonoids from Torreya nucifera displaying SARS-CoV 3CLpro inhibition. Bioorg Med Chem. 2010;18(22):7940–47. doi:10.1016/j.bmc.2010.09.035.
37. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Choe H. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature. 2003;426(6965):450–54. doi:10.1038/nature02145.
38. Simmons G, Reeves JD, Rennekamp AJ, Amberg SM, Piefer AJ, Bates P. Characterization of severe acute respiratory syndrome-associated coronavirus (SARS-CoV) spike glycoprotein-mediated viral entry. Proc Natl Acad Sci. 2004;101(12):4240–45. doi:10.1073/pnas.0306446101.
39. Shanmugaraj B, Siriwattananon K, Wangkanont K, Phoolcharoen W. Perspectives on monoclonal antibody therapy as potential therapeutic intervention for Coronavirus disease-19 (COVID-19). Asia Pac J Allergy Immunol. 2020;38(1):10–18. doi:10.12932/AP-200220-0773.
40. Sui J, Li W, Murakami A, Tamin A, Matthews LJ, Wong SK, Anderson LJ. Potent neutralization of severe acute respiratory syndrome (SARS) coronavirus by a human mAb to S1 protein that blocks receptor association. Proc Natl Acad Sci. 2004;101(8):2536–41. doi:10.1073/pnas.0307140101.
41. Yan Y, Zou Z, Sun Y, Li X, Xu KF, Wei Y, Jiang C. Anti-malaria drug chloroquine is highly effective in treating avian influenza A H5N1 virus infection in an animal model. Cell Res. 2013;23(2):300–02. doi:10.1038/cr.2012.165.
42. Vincent MJ, Bergeron E, Benjannet S, Erickson BR, Rollin PE, Ksiazek TG, Nichol ST. Chloroquine is a potent inhibitor of SARS coronavirus infection and spread. Virol J. 2005;2(1):69. doi:10.1186/1743-422X-2-69.
43. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, Xiao G. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res. 2020;30(3):269–71. doi:10.1038/s41422-020-0282-0.
44. Touret F, de Lamballerie X. Of chloroquine and COVID-19. Antiviral Res. 2020;177:104762. doi:10.1016/j.antiviral.2020.104762.
45. Alves DS, Pérez-Fons L, Estepa A, Micol V. Membrane-related effects underlying the biological activity of the anthraquinones emodin and barbaloin. Biochem Pharmacol. 2004;68(3):549–61. doi:10.1016/j.bcp.2004.04.012.
46. Ho TY, Wu SL, Chen JC, Li CC, Hsiang CY. Emodin blocks the SARS coronavirus spike protein and angiotensin-converting enzyme 2 interaction. Antiviral Res. 2007;74(2):92–101. doi:10.1016/j.antiviral.2006.04.014.
47. Zhang XW, Yap YL. Old drugs as lead compounds for a new disease? Binding analysis of SARS coronavirus main proteinase with HIV, psychotic and parasite drugs. Bioorg Med Chem. 2004;12(10):2517–21. doi:10.1016/j.bmc.2004.03.035.
48. Koch T, Dahlke C, Fathi A, Kupke A, Krähling V, Okba NM, Hesterkamp T. Safety and immunogenicity of a modified vaccinia virus Ankara vector vaccine candidate for Middle East respiratory syndrome: an open-label, phase 1 trial. The Lancet Infect Dis. 2020;20:827–38. doi:10.1016/S1473-3099(20)30248-6.
49. Liu R, Wang J, Shao Y, Wang X, Zhang H, Shuai L, Bu Z. A recombinant VSV-vectored MERS-CoV vaccine induces neutralizing antibody and T cell responses in rhesus monkeys after single dose immunization. Antiviral Res. 2018;150:30–38. doi:10.1016/j.antiviral.2017.12.007.
50. Chi H, Zheng X, Wang X, Wang C, Wang H, Gai W, Xia X. DNA vaccine encoding Middle East respiratory syndrome coronavirus S1 protein induces protective immune responses in mice. Vaccine. 2017;35(16):2069–75. doi:10.1016/j.vaccine.2017.02.063.
51. Hu H, Huang X, Tao L, Huang Y, Cui BA, Wang H. Comparative analysis of the immunogenicity of SARS-CoV nucleocapsid DNA vaccine administrated with different routes in mouse model. Vaccine. 2009;27(11):1758–63. doi:10.1016/j.vaccine.2009.01.021.
52. Hu H, Lu X, Tao L, Bai B, Zhang Z, Chen Y, Wang H. Induction of specific immune responses by severe acute respiratory syndrome coronavirus spike DNA vaccine with or without interleukin-2 immunization using different vaccination routes in mice. Clin Vaccine Immunol. 2007;14(7):894–901. doi:10.1128/CVI.00019-07.
53. Shi SQ, Peng JP, Li YC, Qin C, Liang GD, Xu L, Sun QH. The expression of membrane protein augments the specific responses induced by SARS-CoV nucleocapsid DNA immunization. Mol Immunol. 2006;43(11):1791–98. doi:10.1016/j.molimm.2005.11.005.
54. Woo PC, Lau SK, Tsoi HW, Chen ZW, Wong BH, Zhang L, Chan KH. SARS coronavirus spike polypeptide DNA vaccine priming with recombinant spike polypeptide from *Escherichia coli* as booster induces high titer of neutralizing antibody against SARS coronavirus. Vaccine. 2005;23(42):4959–68. doi:10.1016/j.vaccine.2005.05.023.
55. Zhao P, Cao J, Zhao LJ, Qin ZL, Ke JS, Pan W, Qi ZT. Immune responses against SARS-coronavirus nucleocapsid protein induced by DNA vaccine. Virology. 2005;331(1):128–35. doi:10.1016/j.virol.2004.10.016.
56. Saif LJ. Coronaviruses of domestic livestock and poultry: interspecies transmission, pathogenesis, and immunity. In: Perlman S, Gallagher T, Snijder EJ, editors. Nidoviruses. Washington (DC): ASM Press; 2008. p. 279–98.
57. Jordan B. Vaccination against infectious bronchitis virus: a continuous challenge. Vet Microbiol. 2017;206:137–43. doi:10.1016/j.vetmic.2017.01.002.
58. Liu C, Zhou Q, Li Y, Garner LV, Watkins SP, Carter LJ, Albaiu D. Research and development on therapeutic agents and vaccines for COVID-19 and related human coronavirus diseases. ACS Cent Sci. 2020;6:315–31. doi:10.1021/acscentsci.0c00272.
59. Buchholz UJ, Bukreyev A, Yang L, Lamirande EW, Murphy BR, Subbarao K, Collins PL. Contributions of the structural proteins of severe acute respiratory syndrome coronavirus to protective immunity. Proc Natl Acad Sci. 2004;101(26):9804–09. doi:10.1073/pnas.0403492101.
60. Schindewolf C, Menachery VD. Middle East respiratory syndrome vaccine candidates: cautious optimism. Viruses. 2019;11(1):74. doi:10.3390/v11010074.
61. Mark D. Live attenuated Coronavirus vaccines. US Patent. 2020; US20060039926A1. 2006 Feb 23 [accessed 2020 June 14]. https://patentimages.storage.googleapis.com/b5/d4/fb/5d1a763f553801/US20060039926A1.pdf.
62. WHO, COVID-2019 (World Health Organization). Coronavirus disease (COVID-2019) situation reports. 2020 [accessed 2020

April 22]. https://www.who.int/emergencies/diseases/novel-coronavirus-2019.
63. Smith G, Liu Y, Massare M. Immunogenic middle east respiratory syndrome coronavirus (mers-cov) compositions and methods. WO2015042373A1. 2015 Mar 26 [accessed 2020 June 14]. https://patentimages.storage.googleapis.com/37/79/44/80efd21dd76bd5/WO2015042373A1.pdf.
64. Novavax press release on 2020 Feb 26. http://ir.novavax.com/news-releases/news-release-details/novavax-advances-developmentnovel-covid-19-vaccine.
65. Wu T-C, Hung C-F, Kim TW DNA vaccines targeting antigens of the severe acute respiratory syndrome coronavirus (SARS-CoV). WO2005081716A3. 2005 Sept 9 [accessed 2020 June 14]. https://patentimages.storage.googleapis.com/75/0f/29/7245823f01f88f/WO2005081716A3.pdf.
66. Weiner DB, Muthumani K, Sardesai NY. Mers-cov vaccine. WO2015081155A1. 2015 June 4 [accessed 2020 June 14]. https://patentimages.storage.googleapis.com/76/e3/5b/8215a5a09e4fd0/WO2015081155A1.pdf.
67. Ciaramella G, Himansu S. Respiratory virus vaccines. WO2017070626A2. 2017 Apr 27 [accessed 2020 June 14]. https://patentimages.storage.googleapis.com/e7/f9/bb/eb3ec1e7790856/WO2017070626A2.pdf.
68. Moderna press release on 2020 Feb 24. https://investors.modernatx.com/news-releases/news-release-details/moderna-shipsmrna-vaccine-against-novel-coronavirus-mrna-1273.
69. Baras B, Callendret B, Escriou N, Lorin V, Marianneau P, Werf SVD, Wettendorff MAC. Vaccine. WO2010063685A1. 2010 June 10 [accessed 2020 June 14]. https://patentimages.storage.googleapis.com/3e/71/12/465a5cf4aa8d22/WO2010063685A1.pdf.
70. Humphreys R, Xu M. Ii-key/antigenic epitope hybrid peptide vaccines. US20060002947A1. 2006 Jan 5 [accessed 2020 June 14]. https://patentimages.storage.googleapis.com/a9/80/5b/fb4e65c96b7369/US20060002947A1.pdf.
71. Generex press release on 2020 Feb 27. https://storage.googleapis.com/wzukusers/user-26831283/documents/5e57ed391b286sVf68Kq/PR_Generex_Coronavirus_Update_2_27_2020.pdf.
72. Woelfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Mueller MA, Bleicker T. Clinical presentation and virological assessment of hospitalized cases of coronavirus disease 2019 in a travel-associated transmission cluster. MedRxiv. 2020. doi:10.1101/2020.03.05.20030502.
73. Cheng Y, Wong R, Soo YOY, Wong WS, Lee CK, Ng MHL, Cheng G. Use of convalescent plasma therapy in SARS patients in Hong Kong. Eur J Clin Microbiol Infect Dis. 2005;24(1):44–46. doi:10.1007/s10096-004-1271-9.
74. Ng OW, Tan YJ. Understanding bat SARS-like coronaviruses for the preparation of future coronavirus outbreaks—Implications for coronavirus vaccine development. Hum Vaccines Immunother. 2017;13(1):186–89. doi:10.1080/21645515.2016.1228500.