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Review

SARS-CoV-2 Vaccine Development: An Overview and Perspectives

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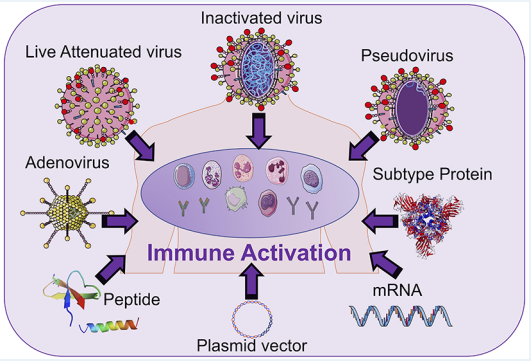
ABSTRACT: Coronavirus disease 2019, abbreviated as COVID-19, is caused by a new strain of coronavirus called severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It started in late December 2019 in Wuhan, China, and by mid-March 2020, the disease had spread globally. As of July 17, 2020, this pandemic virus has infected 13.9 million people and claimed the life of approximately 593 000 people globally, and the numbers continue to climb. An unprecedented effort is underway to develop therapeutic and prophylactic strategies against this disease. Various drugs and vaccines are undergoing rapid development, and some of these are already in phase III clinical trials. Although Russia was the first to release a vaccine by skipping phase III clinical trials, there is no evidence of large-scale clinical trials, and the safety and efficacy of the vaccine are still a concern. Nevertheless, critical lessons can be learned and data garnered for developing promising vaccines against this rapidly emerging virus or other similar pathogens in the future. In this overview, we cover the available information on the various vaccine development initiatives by different companies, the potential strategies adopted for vaccine design, and the challenges and clinical impact expected from these vaccines. We also briefly discuss the possible role of these vaccines and the specific concerns for their use in patients with pre-existing disease conditions such as cardiovascular, lung, kidney, and liver diseases, cancer patients who are receiving immunosuppressive medications, including anticancer chemotherapies, and many other sensitive populations, such as children and the elderly.



KEYWORDS: *COVID-19, SARS-CoV-2, pandemic disease, vaccine, nucleic acid vaccine, antiviral therapy*

1. INTRODUCTION

The COVID-19 pandemic is affecting billions of people around the world. The causative pathogen, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 or SC2), belongs to a family of betacoronaviruses that are enveloped, positive-sense, single-stranded RNA viruses that infect humans and mammals. SC2 shares genomic similarities with SARS-CoV-1 which emerged in 2003 and Middle East respiratory syndrome coronavirus (MERS-CoV) that emerged in 2012.[1] Various findings indicate that SC2 could have likely originated from bats. In addition to bats, this virus can infect cats, ferrets, nonhuman primates, dogs,[2] and humans. This virus uses angiotensin-converting enzyme 2 (ACE2) as a receptor for its cellular entry.[3,4] Although reports from around the globe have noted that most patients with COVID-19 exhibit mild to moderate clinical symptoms, it manifests as a severe disease in about 20% cases, though this varies in different countries.[5−7] As considerable variations are found in the reporting systems for COVID-19, its testing methods, treatment strategies, and patient management, these statistics need further verification and updates to compare the clinical presentations of this infection when correlated to ethnicity and economic status. These issues aside, the case fatality rate (CFR) for COVID-19 shows a strong correlation with age, underlying disease status, and immune conditions. The elderly and those with underlying cardiovascular/cerebrovascular associated diseases (including hypertension, hyperlipidemia, myocardial injury; lung diseases, such as chronic obstructive pulmonary disease; chronic liver disease; chronic renal disease; or those with compromised immune functions) can manifest with severe disease and consequent higher mortality rates.[8−12] The overall COVID-19 infection fatality rate (IFR) among symptomatic cases in the United States was estimated to be ∼1.3% (95% central credible interval: 0.6−2.1%).[13] Populations with undetected, non-syndromic, or mild cases would add significantly to the officially reported cases, thus lowering the reported IRF if the entire population were tested for the presence of the virus or antibody profile (indicating previous exposure to the virus). As of the end of April 2020, the death rate in Italy was a shocking 11%. Meanwhile, neighboring Germany had a mortality rate of just 1%, China had a mortality rate of 4%, and Israel had the

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lowest rate in the world at 0.35%. These rates could be artificial owing to the large number of undetected or asymptomatic nonsyndromic patients, in combination with relatively elderly populations. Meanwhile, the reproductive number of cases (R_0), signifying the cases directly generated by one case to a population, was roughly calculated to be 2−3,[14] which indicates that COVID-19 is more serious than other seasonal influenzas.

Herd immunity has been considered as potentially the most effective way of slowing down the spread of the disease. As an example of natural infection, people who survived the 1918 pandemic were found to be immune to subsequent H1N1 influenza infection.[15] There are two approaches to reach herd immunity: (1) natural infection and (2) vaccines. However, the proportion of the population that has to be immunized to achieve herd immunity varies from disease to disease. It has not been clear to date if infection with SC2 confers immunity to future infections, since an existing case report has described a re-admission of a 41 year old male patient with similar symptoms and imaging findings of COVID-19 after being discharged, 19 days after being considered a "clinical cure".[16] Another study from South Korea reported that 4.5% of patients with severe COVID-19 had been re-admitted after discharge.[17] Somani et al. also reported that 3.6% of COVID-19 patients in New York tested positive again after discharge and were re-admitted to hospital.[18] Moreover, when mutant SC2 variants have been reported[19] and re-infected cases were investigated in different countries, the increasing population of recovered COVID-19 patients might not contribute to effective herd immunity as subsequent waves of the pandemic result from mutant variants of SC2.[20] Despite a recently published nonhuman primate study showing that primary exposure to SC2 can prevent re-infection,[21,22] testing in multiple animal models has led to different levels of confidence in predicting outcomes in humans. For example, studies have shown that some viral-vector and DNA-based vaccines produced promising outcomes only to induce weaker responses in humans.[23] It has been estimated that the COVID-19 pandemic may decrease naturally by establishing high levels of herd immunity, likely in 70% of the population, amounting for more than 200 million people in the United States.[24,25] Nonetheless, a widespread SC2 infection could lead to severe complications and high fatality rates, especially among the elderly and people with chronic diseases. The consequences of broad epidemics could also result in severe burdens on the health care system and, in turn, cause extensive economic disruption. Therefore, development of safe and effective vaccines could be a more straightforward approach that can lead to appropriate degrees of herd immunity to tackle this pandemic disease. Ideally, developing an optimized vaccine capable of protecting against multiple strains of betacoronaviruses would be more efficient, in the anticipation of future outbreaks that might emerge from different types of coronavirus strains.

2. RATIONAL APPROACHES TO DESIGNING A SARS-COV-2 VACCINE PLATFORM

Vaccine development for an acute virus infection is conventionally centered on vaccine-elicited reappearance of phylactic immunity caused by the natural infection. The knowledge of vaccine-assisted phylactic immunity for SC2 is currently limited. SC2 possesses an RNA genome of 29 903 bases reported initially from the NC_045512.2 Wuhan reference genome.[26] They code for various proteins and enzymes which include the structural spike (S) protein, envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein, as well as many other nonstructural proteins.[27] As more sequences become available, according to the data from the Global Initiative on Sharing All Influenza Data (GISAID), the current SC2 viruses are identified as three major clades, G, V, and S, which indicate a variant of the S-protein S-D614G, a variant of ORF3a coding protein NS3-G251, and the variant ORF8-L84S, respectively.[28] Whether differences in characteristics of transmission and mortality rates between different countries are related to the consequence of the different clades in virulence still remains to be determined.[29]

On the basis of our knowledge of the SARS-CoV-1 and MERS-CoV pandemics, the phylactic immune response induced by the virus infection is most likely owed to effective T cells ($CD4^+$ and $CD8^+$) and neutralizing antibody responses specific to the virus.[30,31] Thus, to understand the mechanisms and magnitudes of the specific $CD4^+$ and $CD8^+$ T cell responses to SC2 is of great importance to treatment as well as vaccine selection/development for COVID-19. Most of the information currently used to plan for SC2 treatment, patient management, and vaccine designs is based on the knowledge gained from previous research on SARS-CoV-1 and MERS-CoV. Both SARS-CoV-1 and SC2 infect host cells by targeting ACE2 receptors,[32,33] while MERS-CoV recognizes the dipeptidyl peptidase 4 (DPP4) receptor.[34] SC2 is more closely related to SARS-CoV-1, sharing ∼79.6% genomic sequence homology.[35] Alba Grifoni et al. have identified circulating $CD4^+$ and $CD8^+$ T cell responses in 100 and 70% of patients recovered from COVID-19, respectively. SC2-specific $CD4^+$ T cell response also occurs in 40−60% of unexposed populations, which indicates a cross-reactive T cell identification between common coronaviruses and SC2.[36] The S-protein-specific $CD4^+$ T cell response is likely the most robust and is associated with the magnitude of the IgG and IgA titers against SC2. Additionally, the M and N proteins also account for parts of the total $CD4^+$ responses. The S protein of this virus is the most attractive target for vaccine designs since it is displayed on the surface of the virus and helps the virus infect host cells by binding to cellular ACE2 receptors. In addition, antibodies targeting the ACE2-binding pocket of S-protein could inhibit virus binding with host cells during infection to act as therapeutic antibodies and can elicit neutralizing antibody (nAB) responses against this virus. Several studies have shown a high correlation between the levels of the receptor-binding domain of ACE2−S-protein antibodies and the neutralizing antibodies against SC2 in COVID-19 patients.[37−39] However, there are still differing viewpoints. For example, findings for MERS-CoV from South Korea have suggested that effective immune responses failed to occur, as shown by detection of neutralizing and S-protein antibody titers over a period of 1 year in 11 patients.[40] While most acute viral infections contribute to the phylactic immunity, robust protective immunity may still not be elicited.[41,42]

Before COVID-19, many vaccines have been developed for SARS-CoV-1, including S-protein-based recombinant vaccines, attenuated vaccines, whole inactivated viral vaccines, and vectored vaccines, but only a few of them have progressed beyond phase I clinical trials.[43] Similarly, the early phase clinical trials of MERS-CoV vaccines based on the S protein, such as adenoviral vector expressing S protein, and DNA vectors expressing S protein were slow to succeed in reaching

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later stages of human applications. So far, only one nAb for MERS-CoV has been identified in phase I trials.[44] Hence, the potential for MERS-CoV vaccines to induce cross-neutralizing activity against SC2 needs further validation. All these research outcomes clearly show the difficulties in developing vaccines against the family of coronaviruses and also highlight the strain-specific variations in immune effects that point to the need to focus on SC2 specific antigens to develop vaccines against COVID-19. The vaccine research data from SARS-CoV-1 and MERS-CoV provide some insights and lessons concerning how to move forward with SC2 vaccine design.

Several reports related to SARS-CoV-1 vaccines indicate the protective role of both cell-mediated and humoral immune responses. Early evaluations using DNA and live-attenuated virus vaccines for SARS-CoV-1 generated antibody responses that were protective against virus infection in animal models.[45,46] However, a long-term follow-up study in patients who have recovered from SARS-CoV-1 infection showed a lack of peripheral humoral memory B cells responsive to this virus, which raises concern about a lack of long-term protective immunity.[47] However, T cell responses have been shown to provide long-term protection,[47−49] thus strongly attracting interest for creation of an effective vaccine against SARS-CoV-1.[50] Among all currently known SARS-CoV-1 proteins, T cell responses against the S-protein (when compared to non-structural proteins) have been found to be the most immunogenic in peripheral blood mononuclear cells of convalescent SARS-CoV-1 patients.[51] However, there remains a need to further understand the necessity of antibodies against all structural proteins for long-term and effective immunity. Even after 15 years since its first emergence, we still lack this information for SARS-CoV-1.

When considering widespread vaccination of the healthy population, it is also be important to consider the vast majority of individuals who have impaired immune systems owing to age, autoimmunity, cancer, and other diseases. From the data provided by the Johns Hopkins ABX guide, updated on June 3, 2020, the COVID-19 susceptible population groups that are more prone to develop severe disease include those of older age (>65yrs), especially those with comorbidities, and those aged 20−44 years, who also account for 20% of hospitalizations and 12% of ICU admissions. Although the CDC recently removed the age threshold for the over 65 year old population as a specific susceptible population and warns that all the age groups are at potential risk, the risk does increase with age. Age, therefore, still remains an independent risk factor for severe illness from COVID-19. Consideration of all these factors is important when planning for the development of vaccines. It is important to appropriately design vaccines that are effective in individuals independent of their immune status to elicit antibody responses and to not cause any vaccine-associated pathological side effects, for example, the "immune-enhancement effect" such as (ADE) of viral infection in which the viruses utilize antibodies produced by vaccines to further improve the process of infection rather than showing protective effect.[52] In some cases, this process also helps virus replication into monocytes/macrophages and granulocytic cells through interaction with Fc and/or complement receptors. In addition, T-cell-based immuno-enhancement, which includes allergic responses induced by T helper 2 (Th2)-type immunopathology and excessive cytotoxic T lymphocyte (CTL) response, induces nonspecific cytokine release caused by the absence or insufficient levels of neutralizing antibodies.[53] Additionally, live-attenuated virus vaccines are not recommended for people with immunosuppressed conditions such as infants or cancer patients who are undergoing chemotherapy.[54] Hence, it is paramount to develop alternative vaccine platforms against SC2, which more ideally would also account for other types of coronaviruses and which can protect these populations without showing any adverse effects. Notably, individuals with immune deficiency commonly respond worse to vaccination,[55] but multiple booster doses can achieve protection if the vaccine is considered mild, without vaccine-associated adverse effects. Effective protection against the infection requires higher neutralization titers than in naïve younger adults. Thus, specific strategies for these groups of individuals might require higher doses of antigens or approaches to assist boosting the antigens, i.e., through use of an adjuvant.[56,57]

A recent report by Kuderer et al.[58] summarized the clinical impact of COVID-19 on cancer patients. This multi-institution study showed that increasing age, male individuals, smoking history, underlying comorbidities, and receipt of azithromycin plus hydroxychloroquine have strong associations with 30 day all-cause mortality when compared to the normal population. Hoffmann et al. also showed that TMPRSS2, a frequently altered gene in prostate cancer, may have a strong correlation with SC2 cellular infection by regulation of viral activation and ACE2-mediated host-cell entry, and it can be blocked by a protease inhibitor.[59] Besides TMPRSS2-dependent virus action, regulation of host-cell entry has also been observed for SARS-CoV-1 and influenza H1N1 viruses.[60,61] These findings suggest that mechanisms of COVID-19 infection in cancer patients may be diverse and that strategies for antiviral drug treatment and/or vaccination for cancer patients may be challenging.

As the pandemic continues to expand globally, the number of infected people increases, and more patient data are available. Consequently, several studies have investigated the risk factors and prognosis of COVID-19 infection in populations with underlying cardiovascular and cerebrovascular diseases or/and chronic liver and kidney disease. A study from Wuhan, China, showed that patients with underlying cardiac or/and cerebrovascular disease accounted for more severe outcomes: Intensive care unit (ICU) cases had a risk ratio of 3.30 for cardiac and/or cerebrovascular disease and 2.03 for hypertension when compared with non-ICU cases.[62] Results from the Surgical Outcomes Collaborative registry, which includes 8910 hospitalized, laboratory-confirmed SC2-infected patients from 169 hospitals in Europe, Asia, and America, revealed that patients with a history of cardiovascular disease, such as atherosclerosis, cardiac arrhythmias, or heart failure were significantly associated with higher risks of death.[63] Although there is no tendency for patients with chronic liver/renal conditions to have a greater risk for COVID-19, those with autoimmune liver diseases, liver cirrhosis, hepatocellular carcinoma, or/and underlying renal problems, such as autoimmune renal diseases and postrenal transplant status, may have a higher risk of infection and adverse outcomes. Direct virus-induced damage, infection via ACE2 receptors, systemic inflammatory response induced by cytokines, and immune-mediated damage could also reversely cause liver/renal injury, which may account for the pathogenesis and poor prognosis.[9,64] Lessons learned in that regard could inform other principles applicable in general to COVID-19 vaccine development. A full understanding of the impact of SC2

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Table 1. Major Vaccine Candidates Currently under Development for COVID-19 Disease as of August 2020[a]

developers	vaccine profiles	status	assistive tech
Moderna/CEPI (USA)	LNPs-mRNA vaccine encoding S protein	phase III	
CanSino (China)	Adenovirus type 5 vector expressing S protein	phase III, approved for use in the military	
Inovio/CEPI (USA)	pDNA encoding S protein	phase II/III	delivered by electroporation
Shenzhen Geno-Immune Medical Institute (China)	DCs modified with lentiviral vector expressing synthetic minigene based on domains of selected viral proteins	phase I/II	
Shenzhen Geno-Immune Medical Institute (China)	aAPCs modified with lentiviral vector expressing synthetic minigene based on domains of selected viral proteins	phase I/II	
Johnson & Johnson (USA)	Adenovirus-vectored vaccine using AdVac and PER.C6 technology	phase I/II	
Codagenix (USA)	live-attenuated vaccine	phase I	
University of Queensland/CEPI (Australia)	protein-based vaccine using Molecular Clamp platform	phase I	
Novavax/CEPI (USA)	recombinant nanoparticle technology	phase I/II	
Clover Bipharmaceuticals/CEPI (China)	S-trimer subunit using Trimer-Tag technology	phase I	
Fudan University (China)	S-protein-based mRNA vaccine	phase I/II	
Vaxart(USA)	oral recombinant protein vaccine using VAAST platform	phase I	
CureVac/CEPI (German)	mRNA vaccine	phase I	
BioNTech and Pfizer (German/USA)	mRNA vaccine	phase III	
Takis (Italy)	S-protein-based DNA vaccine	preclinical	delivered by electroporation
Sanofi Pasteur and BARDA (USA)	adjuvanted recombinant vaccine	phase I/II	
Sanofi Pasteur & Translate Bio (USA)	mRNA vaccine	phase I/II	
Sanofi and GlaxoSmithKline (USA)	S-protein based recombinant vaccine	phase I/II	
Hong Kong University/CEPI (China)	replicating viral vector vaccine	phase I	
Oxford University and AstraZeneca/CEPI (UK)	S-protein-based ChAd vector	phase III	
Baxter/CEPI (France)	inactivated vaccine	phase I/II	
Bharat Biotech (India)	inactivated vaccine	phase I	
Madison, Wis. (USA)	S-protein-based recombinant vaccine, cross-protective with influenza	phase I/II	
Sinopharm (China)	inactivated vaccine	phase III	
Sinovac (China)	inactivated vaccine	phase III, collaboration with Butantan (Brazil)	
Generex (USA)	peptide vaccine	phase I/II	
Axon Neuroscience (Slovakia)	S-protein-based peptide vaccine	preclinical	
Gamaleya Research Institute and Russian Defense Ministry (Russia)	S-protein-based Ad vector	approved to use, phase III clinical trials have not yet been completed.	

[a]Abbreviations: aAPC: artificial antigen-presenting cell; DC: dendritic cell; LNP: lipid nanoparticle; S-protein: SARS-CoV-2 spike protein; RBD: receptor binding domain; ChAd: chimpanzee adenoviral; CEPI: Coalition for Epidemic Preparedness Innovations; BARDA: Biomedical Advanced Research and Development Authority.

infection on cancer patients is crucially needed, which would also shed light on specific treatment guidelines, including the use of vaccines for this group of patients. Patients receiving anticoagulant agents, immunoregulatory therapy, antiviral therapy, and chemotherapy need to be given careful consideration when designing and developing vaccines for these specific populations.

3. CURRENT STATUS OF SARS-COV-2 VACCINE DEVELOPMENT

Table 1 and Figure 1 summarize the major confirmed vaccines and approaches that are currently under development for COVID-19, including whole-virus (live-attenuated virus, inactivated virus, and viral-vector) vaccines, subunit vaccines, and nucleic acid vaccines (pDNA and mRNA). As of May 20, 2020, none of these vaccines have been licensed for human applications, but preliminary results in preclinical and/or early phase clinical trials are encouraging global developers to swiftly exploit these approaches at an unprecedented speed to develop and manufacture vaccines for COVID-19.

There are several types of vaccines currently under development for SC2. Here we discuss each vaccine type and the current status of their progress and outcome. Conventional vaccine approaches, such as live-attenuated, inactivated, and subunit vaccines of pathogens, provide durable protection against a variety of dangerous diseases by directly mimicking the natural infection without causing the disease. Live-attenuated vaccines are attractive because the elicited immune response is generally robust, broad in scope, and long-lived; the development of the vaccine does not require *a priori* knowledge of the protective antigens of the pathogen. For example, measles, rubella (MMR), and mumps vaccines and the intranasal influenza vaccine are existing vaccines that are successfully used in human applications. However, live-attenuated pathogens have the potential to revert to a pathogenic form and can cause disease. This is of special concern in children, elderly patients, immunodeficient

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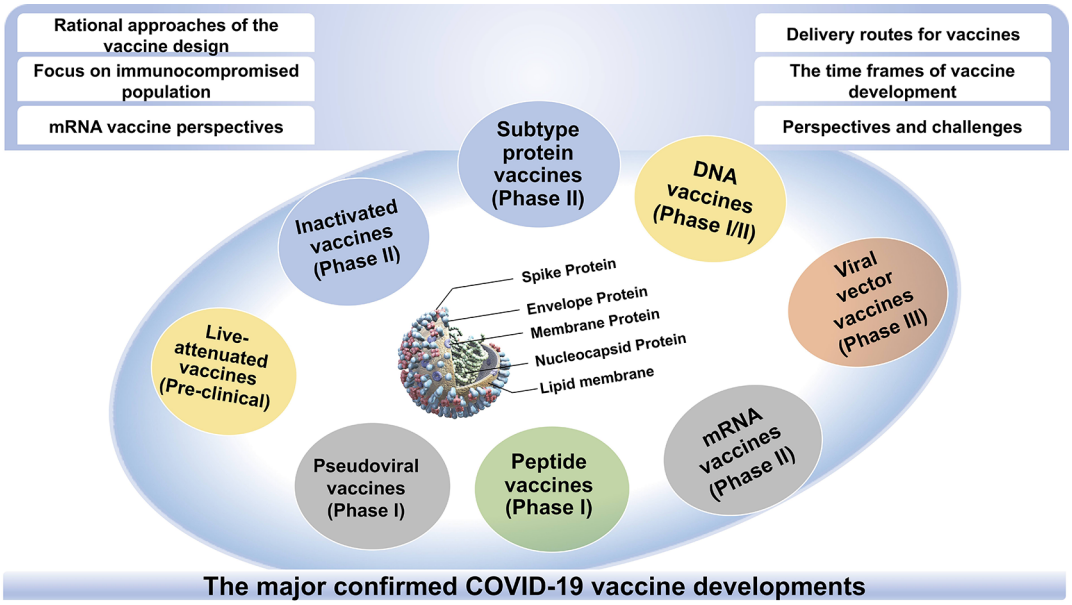

Figure 1. Illustration showing the various types of vaccine approaches currently under development for COVID-19 disease.

individuals, immunosuppressed patients, and cancer patients undergoing chemotherapy. Besides, live-attenuated vaccines are sensitive to temperature and need to be transported and stored under proper conditions. This is difficult in many countries and regions that lack refrigerators. In contrast, inactivated vaccines are made with killed or inactivated germs that produce a prophylactic immune responses in several ways different from those of live-attenuated vaccines. Often, a single dose may not be sufficient to produce or maintain immunity, and multiple doses are necessary. The inactivated polio vaccine is an example of an inactivated vaccine. Subunit vaccines are made using only a part of the viral/bacterial proteins. Subunit vaccines have been developed as a safer alternative, since they only contain the essential antigens, and the side effects are minimal. However, they are less efficient and often require adjuvants. The whooping cough component of the DTaP vaccine is such an example.[65] While live-attenuated vaccines are administered via intravenous, oral, intranasal, and intramuscular routes, inactivated and subunit vaccines are predominantly delivered intramuscularly for effective antigen presentation using slow release. Because SC2 is a new viral strain that is spreading very rapidly and globally, the traditional vaccine development route may not provide an immediate solution. Alternative rapid development and large-scale deployment strategies are needed for COVID-19.

3.1. Whole-Virus Vaccines. In a traditional approach, whole-virus vaccines such as live-attenuated and inactivated virus vaccines are frequently used for immunizing against viral diseases. Lately, with the advanced development of recombinant DNA technologies, pseudoviral particles displaying the antigens of viruses were also explored as vaccines. Live-attenuated vaccines use an attenuated virus or the virus with weakened virulence as a vaccine against the virus. These vaccines, comparable to natural infection, can often create a strong and long-lasting immune response and could provide long-term protection against the pathogen. As schematically shown in Figure 2, viral amplification occurs upon vaccination, which can initiate a strong immune effect compared to that achieved with heat-inactivated and pseudoviral vaccines. For example, the measles, yellow fever, polio, and rotavirus vaccines are the most common live-attenuated vaccines currently used for treatment. However, live-attenuated vaccines have certain limitations. As the delivered vaccine dose entails the use of a small amount of the attenuated live virus, certain groups of individuals, such as those with impaired immune system or/and weakened immunity, and those with chronic health problems or organ-transplant status may need careful consultation with their doctors before receiving these types of vaccines. Besides, these vaccines require strict cold-chain transport, which indicates they may not be used in countries/areas with limited refrigeration capabilities. Unlike a live-attenuated vaccine that uses a weakened form of the virus, the inactivated vaccine uses a killed version of the virus to help protect and create an immune response. The inactivated vaccines may not create immunoprotection that is as strong as that from live vaccines. Therefore, multiple doses would be needed over time with the goal to establish adequate ongoing immunity against diseases. For example, the hepatitis A vaccine and most influenza vaccines are widely used inactivated virus vaccines.

3.1.1. Live-Attenuated-Virus Vaccine. The long-standing strategy of using live-attenuated virus in vaccines has been successful because it shows rapid expansion after injection in the host system. This results an effective immunization strategy when compared to other vaccines without the use of adjuvants or booster doses. Attenuated poliovirus vaccine is the best example of this approach. Codagenix, Inc. (Farmingdale, NY), develops live-attenuated vaccines for different pathogens, such as a live-attenuated-influenza vaccine (LAIV),[66] a respiratory syncytial virus (RSV) vaccine,[67] a tetravalent dengue virus vaccine,[68] and many others.[69,70] The main advantage of a live viral vaccine is its inherent immunogenicity and ability to stimulate toll-like receptors (TLRs). However, it may be problematic to estimate the safety of these vaccines, especially in individuals with immunodeficiency.

3.1.2. Inactivated-Virus Vaccine. Recently, it has been reported that an inactivated SC2 virus vaccine named PiCoVacc showed specific nAB response in mice, rats, and

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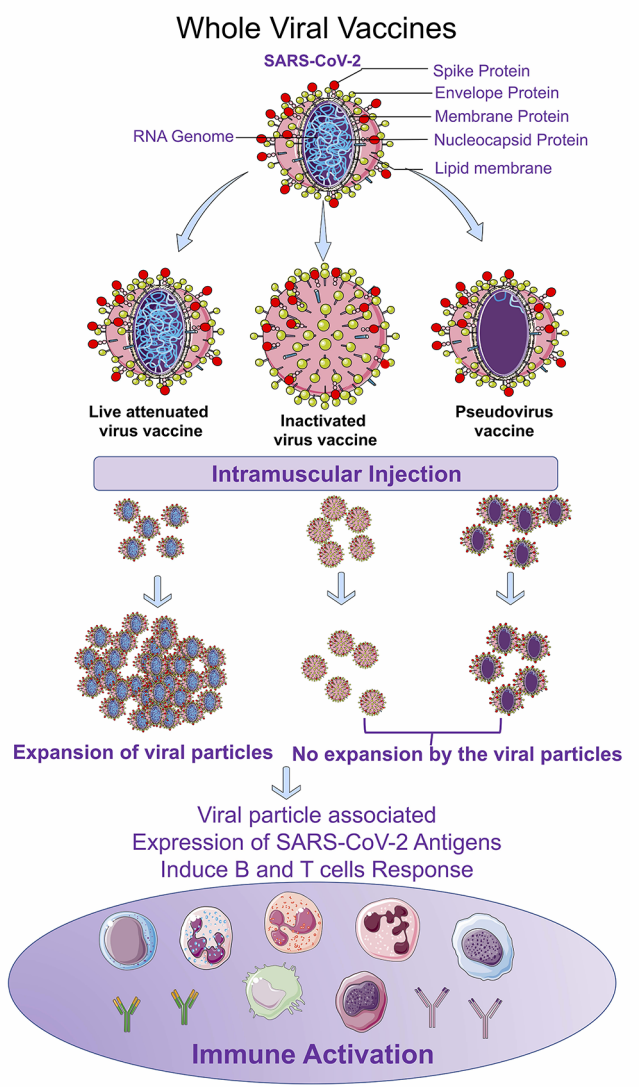

Figure 2. Schematic showing the brief mechanism of immunization using whole-virus vaccines.

nonhuman primates. Three consecutive immunizations using PiCoVacc injected at 3 and 6 μg doses showed partial and complete protection, respectively, against SC2 challenge in macaques.[71] China has approved two inactivated-virus vaccines, one from the Wuhan Institute of Biological Products under the China National Pharmaceutical Group, Sinopharm (Beijing, PRC), the Wuhan Institute of Virology, and the Chinese Academy of Sciences (Wuhan, PRC) and and the other from Sinovac (Beijing, PRC), which are currently in phase II clinical trials.[72] In addition, the Institute of Medical Biology and Chinese Academy of Medical Sciences (Beijing, PRC) have also developed an inactivated-virus vaccine for COVID-19 that is currently in its phase II clinical trial.[73] Another inactivated-virus vaccine, Covaxin, by Bharat Biotech (Hyderabad, India), also entered into human trials in July 2020.[74]

There are, however, a few drawbacks when using whole-virus vaccines, and several reports have highlighted the occurrence of vaccine-associated diseases. For example, an inactivated-virus vaccine formulated in alum against a coronavirus of cats induced an ADE syndrome.[75] Another whole-virus vaccine developed for a respiratory virus in children was found to correlate with vaccine-associated enhanced respiratory disease.[76] There are also reports that inactivated vaccines for RSV infection could cause allergic reaction,[77] which can augment production of multiple inflammatory cytokines (IL-4, IL-5, and IL-13, etc.) that result in increased eosinophil recruitment, mucus production, bronchial hyper-responsiveness, and reduced Th2 cell immune responses. This can be fatal to infants or children with small airways that can be obstructed easily. Thus, in general, whole-virus vaccine development is time-consuming and require extensive testing before they can be deployed for human applications. This is a major issue limiting SC2 vaccine development, given the rapid spread of pandemic COVID-19, thus requiring the speedy deployment of a vaccine(s) for immediate and widespread applications.

3.1.3. Pseudovirus Vaccine. The use of a pseudovirus as a vaccine is another novel concept in vaccine development, where expression of various structural proteins of a virus in transfected/cotransfected mammalian cells using multiple vectors results in assembly of viral particles without loading of the viral genome. This tends to mimic the presence of viruses without the potential to replicate in the host system, as happens with attenuated viral vaccines. Even though pseudovirus vaccines have these theoretical advantages, their production costs and other dose requirements for immunization (when compared to the use of viral structural proteins in expressed protein vaccines) raise concerns as a routine strategy or option for vaccine therapy. Nonetheless, many companies make pseudoviruses using lentiviral and other multivector-based expression systems. They are used as antigens in immune assays and in cell infectivity assays when evaluating safety and immunization effects.[78]

3.2. Viral-Protein-Based Vaccines. Instead of the whole pathogen, viral-protein-based vaccines, for example, subtype protein vaccines and peptide-based vaccines, are the largest categories of vaccines currently under development for various viral diseases, including COVID-19 (Figure 3). Subtype protein vaccines use specific protein fragments of a pathogen that are targeted to key parts of the virus to elicit immune responses. They only contain the essential antigens related to infection, and the side effects are much milder compared to whole-virus vaccines. Protein antigen fragments also contain many antigenic epitopes, some of which may not be necessary (or even deleterious) to eliciting protective immunity. Hence, a "synthetic peptide vaccine" containing only specific epitopes that can induce desirable B- and T-cell-mediated immune responses may be used for immunization. Peptides used in these vaccines usually contain sequences of 20−30 amino acids that are synthesized to produce an immunogenic peptide molecule representing specific epitope(s) of an antigen. These presenting epitopes are the antigenic determinants of the proteins and are thus considered to be sufficient to elicit specific humoral and cellular responses while minimizing reactogenic or allergenic responses.[79] Because of their comparatively smaller sizes, peptides are usually weakly immunogenic on their own and often need carrier molecules to improve their stability, as well as adjuvants to produce robust immune responses.[80,81]

3.2.1. Subtype-Protein Vaccines. Subtype protein vaccines are one of many vaccine types currently under development for COVID-19 that are listed on the GlobalData database, with more than 60 candidates reported to be in phase I trials. Subtype vaccines offer advantages similar to those of whole-peptide vaccine with regard to safety, ease of mass

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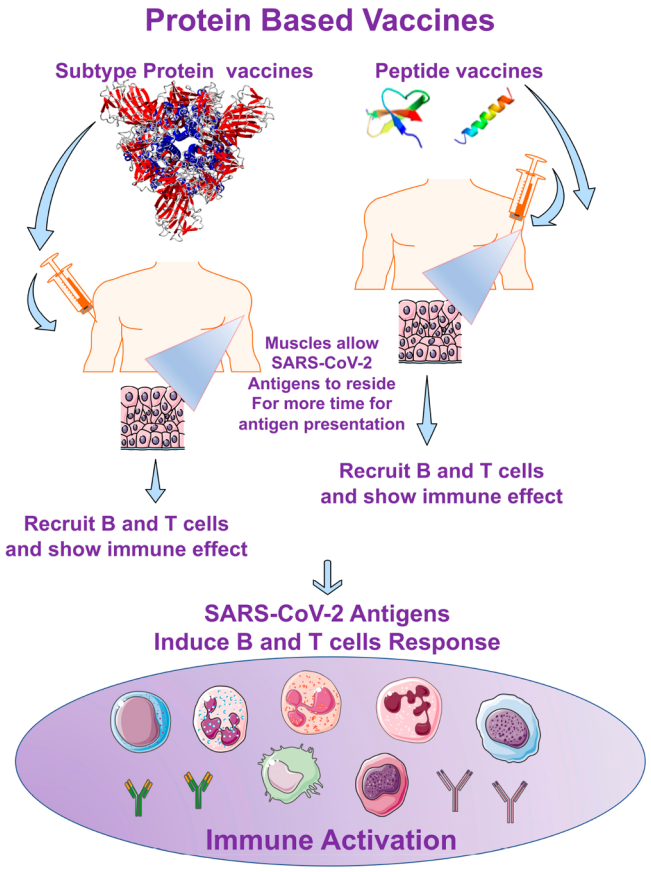

Figure 3. Schematic illustration showing the brief mechanism of the immunization by subtype-protein and peptide-based vaccines.

manufacturing, and precise immune targeting. This approach is based on identification of specific antigens and selection of appropriate delivery systems. Currently, most of the subtype protein vaccines against SC2 mainly target the S-protein to inhibit its binding with the host ACE2 receptor. Since SC2 shares more than 80% amino acid similarity with SARS-CoV-1 and both target the same ACE2 receptor, the hope is that this receptor-binding domain (RBD)-based vaccine has the ability to trigger the same immune reaction as shown for SARS-CoV-1. The University of Queensland has been developing vaccines using viral surface proteins, and the early preclinical testing of one vaccine has shown its ability to raise high levels of antibodies that can effectively neutralize the virus.[82] Novavax (Gaithersburg, TN) and the Melbourne and Brisbane clinics have announced a recombinant S protein nanoparticle vaccine (NVX2373), which is currently reaching phase I/II clinical trials in humans.[83] In addition, a consortium of Clover Biopharmaceuticals (Chengdu, PRC) and Dynavax (Emeryville, CA) is making a trimerized SC2 S-protein based on their Trimer-Tag technology in combination with adjuvants provided by Dynavax (CpG 1018) to develop a subunit vaccine.[84] Moreover, the Texas Children's Hospital Center for Vaccine Development has also developed and is now testing the SARS-CoV-1 and MERS-CoV S-protein subunit vaccines.[85]

3.2.2. Peptide Vaccines. Peptide vaccines are based on the use of peptide fragments to engineer the production of targeted immune responses against specific epitopes of viral antigen. This strategy is attractive because it can help avoid or reduce adverse reactions and allergenic consequences when compared to other conventional vaccines. Axon Neuroscience (Bratislava, Slovak Republic) is developing an S-protein-based peptide vaccine candidate against COVID-19.[86] Generex Biotechnology (Miramar, FL) is currently attempting to develop a promising "complete" peptide vaccine using its Ii-Key technology. The company announced that these Ii-Key-SARS-CoV-2 peptide epitopes, which contain target amino acid sequences from the virus, will be screened against blood samples collected from COVID-19 convalescent (recovered) patients to select those Ii-Key peptides that activate the immune response to fight against COVID-19.[87] Hindawi (London, UK) is working on designing a multi-epitope-based peptide vaccine against the E-protein of SC2.[88] The advantage of using peptide(s) as a vaccine is that clinical-grade peptides can be easily made in large quantities, they are cheap, and are easy to formulate. That said, to our knowledge there currently is no COVID-19 peptide vaccine in clinical trials.

3.3. Nucleic Acid Based Vaccines. As summarized in Figure 4, the application of nucleic acid based vaccines offers a novel approach to immunization and an alternative to many conventional vaccines that induce immune responses similar to those elicited by whole-virus vaccines. Nucleic acid based vaccines initiate the endogenous production of viral antigens that mimic the natural infection of a pathogen. DNA- or RNA-based vaccines have been found to induce both CTL responses and antibody responses to diverse antigens.[89] Many advantages of nucleic acid based vaccines have been recognized, such as the simplicity of the vectors, robust expression and immunogenicity, and the ease of mass production.

3.3.1. Viral-Vector Vaccine. Viral-vector-based vaccine development is another strategy commonly employed in vaccine research. Replication competent and incompetent adenoviral vectors have been commonly used to express the antigens of other viral proteins for immunization. This strategy is quite rapid but limited owing to the immunization effect shown by the adenoviral proteins that are used for the expression of viral antigens of interest as vaccines. The non-replicating adenovirus type-5 (Ad5)-vector vaccine of CanSinoBIO (Tianjin, PRC) was the first SC2 vaccine that reached clinical trials in China.[90] It has now completed a phase II trial and has been approved for use in the Chinese military.[91] The Ad5-vector vaccine has been reported to be immunogenic as early as 28 days after vaccination. Humoral responses against SC2 were detected and peaked at 28 days postvaccination in healthy adults; rapid and specific T cell responses were found from 14 days postvaccination. However, adverse reactions were also noted in some of the participants, which suggested that the Ad5-vector COVID-19 vaccine needs further investigation.[92] Johnson & Johnson (New Brunswick, NJ) is employing the AdVac adenoviral vector of Janssen (Beerse, Belgium) to currently manufacture vaccine for SC2 in their PER.C6 cell line technology, which is originally a vaccine platform developed for the Ebola vaccine.[93] The University of Oxford's nonreplicating chimpanzee adenovirus-vector vaccine (ChAdOx1 nCoV-19) has completed its phase I/II clinical trials and commenced a phase III trial in Brazil on June 20, 2020.[94] Another US company, Vaxart Inc. (San Francisco, CA), has recently initiated preclinical studies for a COVID-19 vaccine using adenovirus vector and is planning for its clinical trials later in the summer of 2020. Their candidate vaccine is based on the company's VAASTT oral vaccine platform, which uses Ad5 as a delivery system for its treatment. They announced on May 20, 2020 that their oral recombinant

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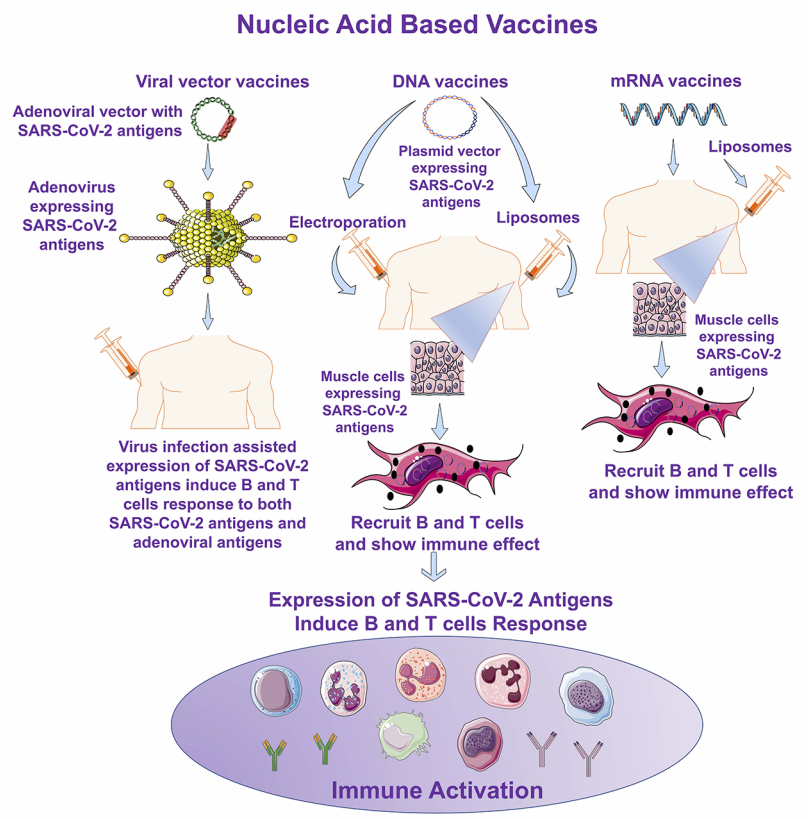

Figure 4. Schematic illustration showing the mechanism of the immunization by different nucleic acid based vaccines in viral diseases.

vaccine, administered in the form of tablets rather than by injection, has been selected as their lead COVID-19 vaccine candidate, and this company has contracted with KindredBio (Burlingame, CA) to manufacture bulk vaccine under cGMP.[95]

3.3.2. DNA Vaccines. The concept of DNA-based immunization began in small animal models in 1993, which showed an immunological protection against influenza, but these findings have not been replicated in humans.[96] More recently, new biotechnological modifications, including delivery formulations, have evolved to improve performances of DNA vaccines. DNA vaccines are designed to express antigens responsible for immunizing against respective organisms. DNA vaccines are attractive because they are easy to formulate and manipulate. They can also be repeatedly injected in multiple-dose immunization schedules to achieve effective antibody responses. A number of biotechnology companies and institutions have reported on advanced nucleic acid vaccine platforms for SC2. Takis Biotech (Rome, Italy) has developed an S-protein-based DNA vaccine which is in preclinical testing. The innovation of Takis consists of the injection of a DNA fragment, followed by a short electrical stimulus called "electroporation" that is expected to amplify the antigen expression by improving DNA transfection.[97] The limitations in the use of DNA vaccines are their inherent property of poor expression owed to poor nuclear transfection and the possibility of the DNA integrating into the genome of cells transfected with the vaccine. Moreover, the practicality or otherwise of using electrical stimulation in human applications is a major concern.

3.3.3. mRNA Vaccines. Recent technological advances in the production of mRNA transcripts of full-length proteins have opened a new area in vaccine development. Currently, there are enormous efforts directed toward developing mRNA vaccines for SC2. The expression of surface antigens (S-, N-, M-, and E-proteins) of this virus using mRNAs is a rapid and safe approach for immediate translation into clinics, because only a few safety issues would be expected. mRNAs can be produced more rapidly, and can be easily standardized. This process improves responsiveness to emerging outbreaks by rapid translation of vaccines to the clinic. Over the past decade, vaccination using *in vitro* transcribed mRNAs encoding viral antigens has become an increasingly promising alternative to pDNA vaccines. The use of mRNA is attractive because of the following: (1) The expression of antigen can be robust and transient. (2) mRNA is noninfectious, and no problems would be expected owing to genomic integration. (3) There is no potential risk of infection or insertional mutagenesis. Additionally, mRNA is degraded by normal cellular processes, and its *in vivo* half-life can be regulated through the use of various base modifications and delivery methods.[98,99] Efficient *in vivo* delivery can be achieved by formulating mRNA into carrier molecules, allowing rapid uptake and expression in the cytoplasm.[100,101] In addition, engineering of the RNA sequence has rendered synthetic mRNAs more translatable than ever before.

*3.3.3.1. mRNA Vaccines for COVID-19*3.3.3.2. Among the various vaccines currently under development for COVID-19, the most likely to be first licensed is the S-protein-based mRNA vaccine (mRNA-1273) from Moderna (Cambridge, USA). According to the phase I/II results just published from Moderna, antibody responses were detected, and the nABs were evaluated in a dose-dependent manner. However, more than half of participants had adverse events including chills, fatigue, headaches, injection site inflammation, and even

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systemic side effects when higher doses (250 μg) of vaccines were administered.[102] The antibody responses did not account for the differences among different populations, including the aged and individuals with underlying conditions. Moreover, the correlation between protection from SC2 and vaccine efficacy in diverse populations remains to be determined in the long term and may be a major challenge for subsequent trials.[102]

To date, efforts are underway to optimize the balance between vaccine efficacy and the proportions of functional components, as well as the exploration of alternative safe and economical materials for multiple antigen-presenting systems. Multiple antigen-presenting systems have been designed for the prevention of cancers and many other infectious diseases, e.g., human immunodeficiency virus,[103,104] hepatitis C virus,[105,106] and SARS-CoV-1.[107,108] These might ideally improve the poor immunogenicity of single antigen-expression vaccines. It would be beneficial for the functional components of a vaccine to contain both B and T cell epitopes to induce strong immune responses rather than only containing B cell epitopes.[109] Full-length mRNA encoding multiple antigen targets plus appropriate delivery systems might be a promising approach. Lipid/polymer nanocarriers, such as cationic lipopolyamines,[110] polyglycolic-*co*-lactides, chitosan, and polymersomes,[111] are proven to act as adjuvants. Lipids derived from bacteria or those containing synthetic α-lipo-amino acids can act as TLR agonists that could recognize structural proteins of viruses. This would enable the secretion of pro-inflammatory cytokines and antibody production by stimulating antigen-presenting cells (APCs).[112,113]

Another S-protein-based mRNA vaccine has been developed by Pfizer (New York) and BioNTech (Mainz, Germany). It was announced on May 8, 2020 that the company has enrolled volunteers for a clinical trial in the NYU Grossman School of Medicine and the University of Maryland. A similar randomized phase I/II clinical trial of this S-protein-based mRNA vaccine was launched in Germany.[91] CureVac (Tübingen, Germany) is developing another mRNA vaccine, currently being evaluated in clinical trials that started in June 2020.[114] The mRNA vaccines for COVID-19 from CureVac and Moderna encode the S-protein to mimic the SC2 infection.

Even though mRNA vaccines offer an effective strategy for antigen expression, their *in vivo* delivery is limited by poor transfection. Although electroporation is a valuable approach, as mentioned earlier, its utility is questionable for clinical applications in humans. Recently, microbubble-ultrasound (MB-US)-aided gene delivery has shown some effectiveness in selectively delivering therapeutic nucleic acids (DNA or RNA) to various tissues *in vivo*.[115] It not only transiently enhances the permeability of vascular tissues but also reduces the thickness of the unstirred layer of the cell surface, thus aiding DNA or RNA entry into cells. It also affects the intracellular vesicles and trafficking after ultrasound exposure, thus enhancing the escape of the nucleic acid cargo from the endosome to the cytoplasm and further transfer to the nucleus (for DNA).[98,116] MB-US mediated delivery of genes and microRNAs in small (mice) and large (pig and dog) animal models of deep-tissue cancers have proven effective.[117−119] This may have some applicability for mRNA vaccine delivery via subcutaneous or intramuscular administration for COVID-19. However, the potential for ultrasound-mediated mRNA vaccine delivery has not been explored, and the immune response induced by MB-US alone is yet to be addressed.

3.3.3.2. Pros and Cons of mRNA Vaccines. A number of sources have indicated that mRNA vaccines may be highly promising because of their safety, flexibility, scalability, and cost-saving.[120] Indeed, mRNA vaccines have great potential and provide many advantages over conventional vaccines. Many preclinical reports have endorsed the application of mRNA-based vaccines.[121] Early clinical trials have also shown the likely tolerability of these vaccines.[102] Notably, mRNA vaccination is capable of inducing antigens specific to T and B cell responses.[122,123] However, the prophylactic efficacy may be low when compared to that observed in animal models.[120,124] Thus, although mRNA vaccines hold great potential, they still require further mechanistic analysis of mRNA action and how this knowledge may be incorporated into creation of novel technologies that can enhance development of mRNA vaccines.

The adoption of novel strategies is also crucially required to render mRNA vaccines effective in other ways, e.g., by decreasing the mRNA dose or the mRNA−carrier dose. The mechanism of immune response induced by mRNA is also not yet fully understood. Furthermore, significant research efforts have been made to enhance the stability and the *in vivo* delivery efficiency of mRNA, its chemical modification of nucleosides,[125−127] and its electroporation-mediated delivery.[128] It has been shown that elimination of the dsRNA contaminant of transcribed mRNAs *in vitro* may prolong the translation.[129] Furthermore, chemically modified nucleosides have reduced the innate immune responses and improved the specific-antigen expression. Another strategy, optimizing the 5′-UTR of mRNA, could also enhance the translational efficiency of mRNA.[130,131] Lastly, a high level of transgene expression using mRNA electroporation was observed in dendritic-cell-based tumor vaccines, perhaps warranting further investigations for adaptation to COVID-19 vaccination.[128,132]

Despite recent reports that early clinical results are promising and will hopefully establish scalable production, mRNA vaccine technology still requires further optimization and more extensive clinical testing. The optimization of these vaccines should take into account the effects of multiple-antigen-specific immune responses, as well as memory cell immune responses that include T and B cell responses.

4. THE EXPECTED TIME FRAME FOR VACCINE DEVELOPMENT

Currently, there are no licensed vaccines available for COVID-19. Some novel technologies used in the platforms for production of COVID-19 vaccines need to be verified for their long-term impact before they can be used in human applications. The vaccines generated under good laboratory practices (GLPs) need to be tested in preclinical animal models, which might take several months to complete. For some SC2 vaccine platforms, portions of safety tests were skipped, as sufficient data were available for vaccines made in the same development process.[133] Drugs and vaccines for human use need to be produced in compliance with good manufacturing practice (cGMP) to ensure persistent quality and safety before they can be used for clinical applications. Currently, most of the proposed vaccine candidates for COVID-19 are still in the preclinical phase or in early clinical trials. The process of cGMP, which is lengthy, has not started yet. Once adequate preclinical and clinical trial data are available, the production of cGMP-quality vaccines can be scaled up for human applications. Typically, clinical tests for

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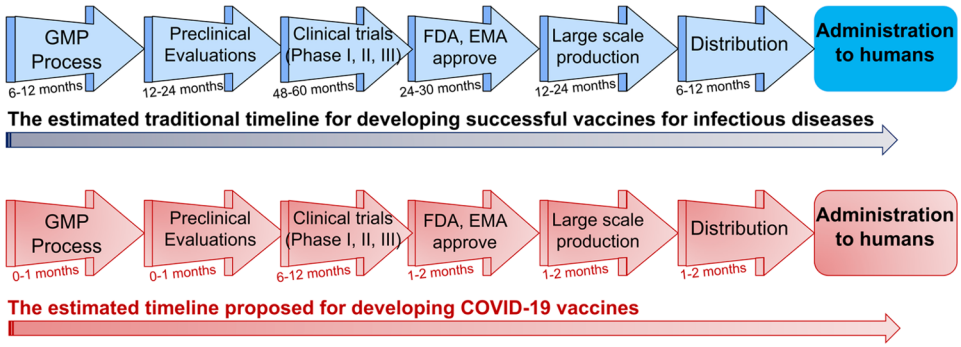

Figure 5. Comparison of the time line of classical vaccine development for infectious diseases versus the time line proposed for COVID-19 vaccines.

vaccines start with phase 0 volunteers followed by small-scale phase I trials. These are followed by phase II clinical trials and ultimately by phase III trials, in which the safety and efficacy of the vaccine would be analyzed in a larger cohort. Finally, it will take time to physically distribute the vaccines to the geographic areas affected by the infection. The workflow in vaccine development process is briefly summarized in Figure 5.

5. PERSPECTIVES AND CONCLUSIONS

As shown in Figure 5, the global efforts in research and development for COVID-19 vaccines have been unprecedented. The experiences gained from SARS-CoV-1 and MERS-CoV research results have been applied to some extent to SC2 vaccine development. Attempts at production of a COVID-19 vaccine have evolved rapidly, and the first clinical trial of an SC2 vaccine began within 4 months of the first outbreak of the virus. As of mid May 2020, a number of pharmaceutical and biotechnology companies have enrolled or even finished their phase I/II clinical trials. It is known that some vaccine platforms have been initiated without prior demonstration of efficacy in preclinical animal models. The swift spread of the pandemic has changed the conventional order of vaccine development and pushed companies to skip some of the development steps as they weigh this against the importance and impact of the disease.

The long-term solution to the infection caused by the novel SC2 virus is the development of safe and effective therapeutics and vaccines. New strategies are needed to produce vaccines that could initiate wider heterologous and/or cross-protective immunity against coronaviruses. This might require a focus on additional viral proteins (N, E, M, etc.) that could induce broader cross-protective immunity. Meanwhile, certain populations remain of concern if they receive vaccines, such as the elderly, younger children, cancer patients, pregnant women, and individuals with chronic diseases or with immunocompromised conditions. It is possible that some types of vaccines or modes of administration might be suitable for these categories of individuals, such as the use of adjuvants that could amplify immunogenicity, thus making lower doses possible. This could enable vaccination of more individuals without compromising safety. Lipid nanoparticles (LNPs) have been widely explored and used as vectors for loading mRNA/pDNA to create a novel nucleic acid vaccine platform (that can also act as an adjuvant). They are typically composed of PEGylated-lipid, cholesterol, ionizable lipid, and an assistant lipid such as distearoylphosphatidylcholine (DSPC).[134] The most clinically advanced LNPs contain the ionizable lipid MC3, lipofectamine, as well as lipid-poly(lactic-*co*-glycolic acid) hybrids.[135,136] However, the LNPs/RNA ratio might need to be 10:1 to ~30:1;[137] thus, to achieve higher amounts of encoded antigens, a large amount of LNPs would be required for any given dose. However, this would also raise questions of safety and tolerability, since LNPs are known to have inherent degrees of immunogenicity and certain levels of toxicity.[138] Accordingly, these issues should be addressed during the development of an SC2 vaccine by optimizing the benefits while reducing the risks, especially for vulnerable individuals. Thus far, more than 10 developers have suggested plans to create adjuvanted vaccines against SC2. In addition, electrical stimulation has been reported by Takis Biotech to assist delivery of DNA-based vaccines, which could be expected to achieve greater immune protection with lower dosage of vaccines. Moreover, we look forward to exploiting the application of MB-US in the study of nucleic acid vaccine delivery and expect such a strategy could be efficient in expanding the immune protection effect. This may provide more alternatives to adoption for nucleic acid based vaccine platforms and greater choices for specific populations. In addition, through the study of cancers and analysis of the characteristics of COVID-19 infection in cancer patients, more therapeutic targets interfering with SC2 infection will be further considered, e.g., TMPRSS2, a highly altered gene in prostate cancer, which is dependent on SC2 cellular infection, and the cell-entry pathway could be diminished by a clinically proven protease inhibitor. These findings also provide us with a new way to think about vaccination strategies for cancer patients.

The somewhat accelerated strategies that are being applied to COVID-19 vaccine development include identification of new viral targets and the use of novel vaccine technology platforms. However, doing so at a speed to match that of the pandemic might also increase the risks associated with hurried vaccine development. The extensive tests and careful evaluations of safety and efficacy at every single step are critically important. Additionally, SC2-specific animal models, such as ACE2-transgenic mice, hamsters, ferrets, and nonhuman primates, need to be developed in order to better evaluate the efficacy of vaccines in preclinical models, and to allow for rapid clinical translation. Ultimately, extensive international collaboration between public health institutions, vaccine developers, funding entities, and governments will be critical and necessary to ensure effective manufacturing of licensed vaccines in adequate quantities is available to all individuals afflicted by COVID-19 in affected areas. Challenges still exist

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despite the remarkable progress being made in the development of vaccines around the world. There are many issues that remain to be learned and resolved about novel SC2, including its biological properties, epidemiology, and other yet-to-be-determined challenges, which can all arise during future phases of COVID-19 vaccine development and evaluation.

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