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MAYO CLINIC

SARS-CoV-2 Vaccine Development: Current Status

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Abstract

In the midst of the severe acute respiratory syndrome coronavirus 2 pandemic and its attendant morbidity and mortality, safe and efficacious vaccines are needed that induce protective and long-lived immune responses. More than 120 vaccine candidates worldwide are in various preclinical and phase 1 to 3 clinical trials that include inactivated, live-attenuated, viral-vectored replicating and nonreplicating, protein- and peptide-based, and nucleic acid approaches. Vaccines will be necessary both for individual protection and for the safe development of population-level herd immunity. Public-private partnership collaborative efforts, such as the Accelerating COVID-19 Therapeutic Interventions and Vaccines mechanism, are key to rapidly identifying safe and effective vaccine candidates as quickly and efficiently as possible. In this article, we review the major vaccine approaches being taken and issues that must be resolved in the quest for vaccines to prevent coronavirus disease 2019. For this study, we scanned the PubMed database from 1963 to 2020 for all publications using the following search terms in various combinations: *SARS*, *MERS*, *COVID-19*, *SARS-CoV-2*, *vaccine*, *clinical trial*, *coronavirus*, *pandemic*, and *vaccine development*. We also did a Web search for these same terms. In addition, we examined the World Health Organization, Centers for Disease Control and Prevention, and other public health authority websites. We excluded abstracts and all articles that were not written in English.

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Severe acute respiratory syndrome coronavirus (SARS-CoV) 2 is the second virus to cause a human pandemic in the 21st century and the third novel betacoronavirus to emerge as a human pathogen in the past 18 years.[1] As of this writing, over 13 million cases have been identified worldwide, and almost 600,000 deaths have been reported.[2] These numbers are certainly underestimates, in part related to the now-apparent wide spectrum of disease ranging from asymptomatic individuals to severe disease to death, as well as the dearth of diagnostic testing and standardized reporting. In a mere 28 weeks, we have learned much, but far more remains to be learned. The genetic sequence of SARS-CoV-2 was solved very quickly, and within weeks the sequence was widely available, identifying it as a betacoronavirus with close genetic similarity to SARS-CoV-1. We have learned the cellular receptor used by this virus and the concomitant inflammatory cytokine storm that can result from infection. We have identified the approximate reproductive number and the infection fatality rate of SARS-CoV-2 and are witnessing the wide spectrum of clinical and human immune responses to infection with the virus. We have also learned that severe disease and death vary by patient age, comorbidities, smoking status, body mass index, when in the context of a localized epidemic one presents for medical care, and other factors. At this time, there are no validated point-of-care assays for rapid diagnostics that have been widely deployed, no licensed antivirals, and no licensed vaccines for use in civilian populations. Absent a safe and effective vaccine, safe achievement of herd immunity will prove elusive. Although multiple vaccine candidates were developed against the

SARS-CoV-1 and Middle East respiratory syndrome (MERS) viruses, no vaccine candidates progressed past phase 1 studies. This review will focus on the current development of SARS-CoV-2 vaccines and address issues relevant to devising a safe and effective vaccine. To gather material for this review, we scanned the PubMed database from 1963 to 2020 for all publications using the following search terms in various combinations: *SARS*, *MERS*, *COVID-19*, *SARS-CoV-2*, *vaccine*, *clinical trial*, *coronavirus*, *pandemic*, and *vaccine development*. We also did a Web search for these same terms. In addition, we examined the World Health Organization (WHO), Centers for Disease Control and Prevention (CDC), and other public health authority websites.

ARTICLE HIGHLIGHTS

- This review briefly summarizes what is currently known about severe acute respiratory syndrome coronavirus 2 and outlines the implications that knowledge may have on vaccine development.
- This review summarizes current coronavirus disease 2019 (COVID-19) vaccine approaches and issues that must be resolved as we work toward developing safe and effective vaccines to prevent COVID-19.
- In particular, the vaccines in advanced phase 3 clinical trials are reviewed, and we outline the rationale for their use.
- We describe potential challenges and a research agenda critical to COVID-19 vaccine development.

SARS-COV-2 DISEASE/OUTBREAKS

In December 2019, a cluster of atypical viral pneumonia cases in Wuhan, China, was reported to the WHO. It was reported that most patients exhibited respiratory symptoms consistent with severe acute respiratory disease (SARS) and had previously visited the Huanan seafood wholesale market, suggesting an animal origin of SARS-CoV-2. Subsequently, person-to-person transmission through droplets or direct contact occurred, resulting in the early January 2020 declaration by Chinese health authorities that a novel coronavirus (2019-nCoV) had been identified and isolated from patients in Wuhan.[3-5] Within a month, the full-length genomic sequence of the new coronavirus was made available to the WHO and became publicly available.[6]

SARS-CoV-2 has rapidly spread across the globe.[7] On January 30, 2020, the WHO declared the SARS-CoV-2 epidemic a global health emergency. By March 11, 2020, the WHO declared a pandemic. The rapid transmission of SARS-CoV-2 has caused fear, panic, economic disruption, morbidity and mortality, and significant public health concerns.[8] Recent data have revealed that the clinical characteristics of coronavirus disease 2019 (COVID-19) can be very heterogeneous with a broad spectrum of severity, including illness resulting in death.[9] Data document that asymptomatic individuals can transmit SARS-CoV-2 infection.[10] Individuals over 65 years of age, individuals of all ages who have serious underlying medical conditions, and those who are immunocompromised are at higher risk of serious COVID-19 illness and complications. Although COVID-19 has been detected in equal numbers of confirmed cases in males and females, there appear to be sex-based differences in severity and mortality of the disease (ie, higher mortality in older male patients).[11,12] Considering the alarming outcomes of the current COVID-19 pandemic, it is critical to develop safe and effective vaccines and antiviral agents to prevent, control, and treat COVID-19.[13]

The SARS-CoV-2 virus shares up to 82% nucleotide identity with human SARS-CoV-1 and utilizes the same host cellular receptor as SARS-CoV-1, angiotensin-converting enzyme 2 (ACE2),[14] as an entry receptor into host cells.[15] Data from initial COVID-19 studies revealed that ACE2 is differentially expressed in many human tissues, such as lung (type 2 alveolar cells), liver (cholangiocytes), stomach (epithelial cells), ileum (enterocytes), kidney (proximal tubules), and colon (colonocytes).[16] Notably, the cellular serine protease TMPRSS2 (also a host cell factor for influenza A and other coronaviruses) has recently been documented as critical for activation of the SARS-CoV-2 transmembrane spike (S)

glycoprotein (ie, the main target of neutralizing antibodies), priming, and viral cell entry.[17,18] Wang et al[19] found that SARS-CoV-2 can invade host cells (Vero E6 cells) via a new CD147-S protein route. This observation indicates that CD147 receptor-targeted antivirals might also be a useful therapeutic strategy against COVID-19.

Little is known about SARS-CoV-2—specific immune responses during COVID-19 infection. Histopathology, immunohistochemistry, in situ hybridization, and electron microscopy data from SARS-CoV-1—infected human lung tissue revealed that SARS-CoV-1 can infect and replicate in alveolar macrophages, type I and type II pneumocytes, and bronchiolar epithelial cells.[20,21] Both Th1-type (interferon-γ, interleukin [IL] 1β, inducible protein 10, monocyte chemotactic protein 1, and IL-6) and Th2-type (IL-4 and IL-10) cytokines are produced in high concentrations in plasma in response to SARS-CoV-2 infection, indicating that the host immune response itself is involved in disease progression and pathogenesis.[22]

In silico study results reveal that SARS-CoV-2 S protein induces an innate inflammatory immune response via nuclear factor κB activation and possibly through Toll-like receptor (TLR) 4 ligand.[23] High concentrations of proinflammatory and anti-inflammatory cytokines (eg, IL-2R, IL-6, IL-10, and tumor necrosis factor α) have been detected in serum samples from severe cases of COVID-19 compared with levels in serum from moderate cases. This finding suggests that a massive cytokine storm likely contributes to disease severity.[24] Other factors that have been reported to be associated with disease severity outcomes (eg, lymphopenia, decrease in $CD4^+$ and $CD8^+$ T lymphocyte counts, suppressed interferon-γ secretion by $CD4^+$ T lymphocytes, and lower counts of $CD16^+CD14^+$ monocytes) may also be potential significant immunologic markers of severe and moderate COVID-19.[24,25] As per a recent case report, the increased frequency of antibody-secreting cells, follicular helper T cells, activated $CD38^+$ HLA-DR^+ $CD8^+$ and $CD4^+$ T lymphocytes, together with SARS-CoV-2—specific IgG and IgM antibodies, detected in the blood of a patient with nonsevere COVID-19 prior to symptomatic recovery, suggests that early adaptive immune-related biomarkers may be predictors of better clinical outcomes.[25] Given SARS-CoV-2 pathogenesis and tissue tropism, and the significant morbidity and mortality at the public health level, it is essential to develop an effective vaccine to protect against SARS-CoV-2.

SARS-COV-2 VIRUS

SARS-CoV-2 is an emerging, enveloped, nonsegmented, approximately 30-kilobase, positive-sense RNA virus of global significance. It belongs to the subfamily Orthocoronavirinae, in the family Coronaviridae (group betacoronavirus).[26,27] Among coronaviruses that can infect humans, 6 types have been previously described: alphacoronaviruses HCoV-229E and HCoV-NL63 and betacoronaviruses HCoV-OC43, HCoV-HKU1, SARS-CoV-1, and MERS coronavirus.[28] Current evidence demonstrates that SARS-CoV-2 and MERS coronavirus are highly transmissible, pathogenic, and associated with significant morbidity and mortality in humans.[7]

Phylogenetic analysis has indicated that SARS-CoV-1, MERS coronavirus, and SARS-CoV-2 most likely originated from bats, with transmission to human populations happening via intermediary animal hosts.[29] Genome composition studies, which have yielded significant insights into the divergence of SARS-CoV-2, resulted in the identification of 380 amino acid substitutions between amino acid sequences of SARS-CoV-2 (Wuhan/HB01 strain) and the equivalent sequences of SARS-CoV-1.[26] A recent phylogenetic network study of 160 SARS-CoV-2 genome nucleotide sequences from COVID-19 cases around the world identified multiple mutations in SARS-CoV-2 viral genomes (ie, nonsynonymous C28144T, synonymous T29095C, and synonymous T8782C), which may help track COVID-19 infection sources.[30] Examination of 247 sequences of SARS-CoV-2 genomes found 4 viral clusters demonstrating a high mutation rate and each becoming prevalent in various countries.[31] It is imperative to understand if and how nonsynonymous and synonymous

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variations, as well as recombination events in the SARS-CoV-2 genome, alter viral binding to the ACE2 receptor, affect virulence, alter transmissibility, or potentially alter the efficacy of antivirals, monoclonal therapeutic antibodies, or vaccines. The estimated mutation rate in the SARS-CoV-1 genome appears to be moderate (0.80 to 2.38 $\times$ 10^{-3} nucleotide substitution per base per year).[32,33] The SARS-CoV-2 genome exhibits a mutation rate of less than 25 mutations per year, which is much slower than seasonal influenza virus, but it is virtually certain that further mutations and recombination events will be identified.

The receptor-binding domain (RBD) of the S protein is a critical factor for binding to ACE2 and to determine tropism and infectivity of SARS-CoV-2.[14] Previous mutagenesis studies have suggested that the cross-neutralization resistance between SARS-CoV and palm civet-CoV may result in mutations within the RBD of the S protein.[34] Recent structural analysis of S protein-ACE2 receptor complexes identified several amino acid substitutions and deletions in the SARS-CoV-2 RBD of S protein (ie, S1 subunit) compared with those of SARS-CoV and bat coronavirus.[35] These mutations resulted in a higher affinity of the SARS-CoV-2 S protein for the human ACE2 in comparison with SARS-CoV and bat coronavirus.[35] This difference is likely associated with the dynamic of viral spreading. Another genetic study examining amino acid mutations in circulating SARS-CoV-2 RBDs has found 8 mutation types (from a total of 18 mutant strains) that were divided into 2 different groups of amino acid mutations in SARS-CoV-2 RBDs based on human ACE2 affinity for the S protein (ie, the "similar affinity" group—V341I, F342L, R408I, A435S, and V483A—and the significantly "higher affinity" group—N354D, D364Y, V367F, and W436R).[36] The study investigators proposed that the "higher affinity" group of mutated amino acids (specifically, amino acid mutation V367F) demonstrated an enhancement of SARS-CoV-2 binding affinity to human ACE2 and may have allowed for increasingly significant infectivity and more severe virus transmission.[37] A pipeline data analysis of real-time mutations in SARS-CoV-2 identified 14 mutations in S protein (including mutation D614G) and a viral recombination event. Recent reports have revealed that the D614G mutation increases infection of human cell lines,[38-40] with mounting evidence that it also influences disease severity.[41]

A study by Fehr and Perlman[42] found that the human SARS-CoV-2 genome is similar to that of other RNA viruses and encodes for 4 major structural proteins, including the surface S, small envelope (E), membrane (M), and nucleocapsid (N) proteins. The coronavirus genome also encodes for 5′ nonstructural (n=16) and lineage-specific accessory genes (n=6, functionally not well characterized). The betacoronaviruses HCoV-OC43 and HCoV-HKU1 have also been found to encode for an additional structural hemagglutinin esterase protein that may enhance the S protein—mediated viral infection and generation of infectious virions.[43] The S protein is implicated in host cell invasion and is cleaved by furin-like enzymes into 2 functional subunits or regions, S1 and S2, which are responsible for host cell receptor binding and host receptor membrane fusion, respectively.[44,45] The S protein of SARS-CoV-2 is primed/activated by the cellular serine protease TMPRSS2, which is essential for viral entry and spread in the infected host.[17] The main factor determining SARS-CoV-2 tropism is the RBD of S protein, which binds to the host receptor ACE2 and can only interact with the RBS when it is in the hinge-like "up" conformation in SARS-CoV-2.[46] Notably, SARS-CoV-2 and SARS-CoV share a conserved epitope in the RBD that may be an important consideration in SARS-CoV-2 vaccine antigenicity and cross-protective antibody responses. This theory remains to be further elucidated.

ISSUES IN VACCINE DEVELOPMENT

The WHO is coordinating an international group of experts (eg, scientists, physicians, and industry leaders) who are working to create vaccine candidates and has released a target product profile[47] that includes both critical and preferred characteristics—the

TABLE 1. Ideal SARS-CoV-2 Vaccine Characteristics[a]

The ideal vaccine should

- Have an excellent safety profile across multiple population groups (eg, children, older adults, pregnant women, immunocompromised individuals)[b]
- Have no contraindications[b]
- Have minimal adverse events that are mild and transient[b]
- Be suitable for administration to all ages, including pregnant women[b]
- Induce protective immunity—ideally after a single dose[b]
- Generate protective immunity rapidly, ideally within 2 weeks[b]
- Have at least 70% efficacy[b]
- Not elicit immunopathology (after vaccination or after subsequent infection) or evidence of antibody-enhanced disease
- Induce protection in health care workers who may face high-titer virus exposures
- Provide long-lasting protection involving both humoral and cell-mediated responses that last for at least 1 year[b]
- If booster vaccinations are needed, preferably require them no more frequently than yearly[b]
- Be quickly mass produced
- Be stable at room temperature to avoid cold chain and transportation issues and facilitate distribution and availability[b]
- Be administered through mechanisms that do not require highly trained health care professionals
- Have the potential for coadministration with other vaccines[b]

[a]SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2; WHO = World Health Organization.

[b]Indicates a characteristic included in the WHO target product profile.[47]

ideal vaccine will have the features outlined in Table 1. The US Food and Drug Administration has recently issued a guidance document on COVID-19 vaccines.[48] This document is primarily focused on regulatory requirements and key considerations for licensure data such as the following: preclinical data, characterization of immune responses in animal models, toxicity, minimum efficacy requirements, and the potential for vaccine-associated enhanced respiratory disease. Despite the relatively mild disease seen in most infants and children and given the relative ease of transmission of SARS-CoV-2, vaccines targeting this audience must also be developed.

It is likely that multiple vaccine candidates, each geared toward specific population groups at increased risk, will be necessary. Because we have never had a coronavirus vaccine, it is also likely that we will need long-term data on multiple vaccines in order to identify products that meet most of those desired characteristics. This process requires thoughtful consideration and collaborations such as that proposed by the Accelerating COVID-19 Therapeutic Interventions and Vaccines public-private partnership.[49] Ethical considerations regarding vaccine testing in the elderly, in children, in pregnant women, and in other vulnerable populations must also be carefully considered. To accelerate vaccine development, calls for human challenge models have also emerged. Thus far, a clear and compelling ethical framework for making such decisions has not reached consensus.

Data from studies on SARS and MERS vaccine candidates have shaped much of the early vaccine development efforts to SARS-CoV-2.[50] Preclinical studies, animal models, and other data have been used to accelerate vaccine development for SARS-CoV-2. These data indicate the following: (1) the S protein is the major target of neutralizing antibodies,[51] (2) many of these antibodies target the RBD of S protein,[52-54] (3) neutralizing antibodies generated by vaccination or adoptively transferred are protective in animal models (eg, mice, rabbits, and nonhuman primates),[55,56] (4) clinical trials of 2 MERS vaccines, a DNA-based vaccine consisting of the S protein and a replication-deficient chimpanzee adenovirus expressing the S protein, both elicit robust antibody responses, (5) a modified vaccinia virus Ankara (MVA)—based vaccine expressing the S protein has been used to vaccinate camels and significantly reduces viral loads and virus secretion,[57] and (6) most of the vaccine candidates also induced cellular immunity, which is thought to be critical to viral clearance.[58-60] Work in these areas continues with an expanded scope that now includes this latest novel coronavirus.

This same body of work with SARS and MERS has revealed that there are also obstacles that we must carefully navigate. One serious issue is antibody-dependent enhancement (ADE) of infection and disease that has been noted in SARS.[61] Interestingly, antibodies targeting the S protein were found to mediate ADE,[62] which results in enhanced infection of macrophages and B cells[63,64];

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therefore, S only protein-based vaccines must be carefully evaluated in terms of safety. Another obstacle facing vaccine development is that animal studies of SARS and MERS vaccines (including formulations that moved into phase 1 clinical trials) found evidence of lung and/or liver pathology after live-virus challenge.[65-68] Eosinophilic infiltration, enhanced Th2 responses, and increased infectivity have been noted with both whole-virus vaccines and with full-length S protein—based vaccines.[69] The ability of a vaccine to elicit a robust, Th1-type helper T cell response is considered ideal, given the antiviral properties of this type of response and its suppressive effect on Th2 responses.[70] Yet another issue is the observed lack of durable protective immunity to seasonal coronaviruses. It remains to be seen if the appropriate use of adjuvants and highly immunogenic vaccination platforms are able to overcome this problem. A number of studies have begun to examine immune responses to SARS-CoV-2 and have found that antibody responses (IgM, IgG, IgA) appear 1 to 2 weeks after infection, peak several weeks later, and then decline. Humoral immunity targets the S and nucleocapsid proteins, with neutralizing antibody primarily directed against the RBD of the S protein.[71-76] Similarly, infection induces T-cell responses (primarily Th1) against a broad range of viral proteins.[77-82] It has also been shown that follicular helper T cell responses occur and are correlated with the magnitude of the humoral response.[83] T-cell responses have also been detected in individuals lacking humoral immunity.[84] Despite these initial findings, our understanding of SARS-CoV-2 immunity is far from complete; therefore, further studies examining innate, humoral, and cellular immune responses to this new virus are necessary in order to fully understand mechanisms of protection that need to be activated by COVID-19 vaccines.

COMPUTATIONAL APPROACHES TO ACCELERATE VACCINE DEVELOPMENT

Because the immune response to SARS-CoV-2 has not yet been fully characterized, we have a very limited understanding of the viral proteins that may be important targets of the immune system, which could be useful for developing effective vaccine candidates. In this respect, predictive computational algorithms may prove to be beneficial tools for the identification of immunogenic T-cell and B-cell epitopes that can accelerate the rational design of SARS-CoV-2 vaccine formulations. Computational algorithms offer the distinct advantage of rapidly screening the entire amino acid sequence of viral proteins to predict peptides with high antigenicity or binding affinity for HLA molecules—a task that would take countless hours to accomplish in the laboratory while consuming valuable biological specimens. Advancements in machine learning, artificial neural networks, and other computational fields have led to the continued development and refinement of epitope prediction algorithms with improved accuracy,[85-94] but performance gaps still exist. Studies of vaccinia virus infection have found that computer-based algorithms fail to identify up to 20% of peptides presented by HLA molecules,[95] and the conformational nature of B-cell epitopes makes it difficult for computer-based methods to accurately predict them. Nevertheless, these approaches are state-of-the-art for epitope identification in the absence of biological data and, given the urgent need for a vaccine to combat the spread of COVID-19, should be used to guide experimental vaccine design where appropriate.

Computational algorithms have been previously applied for the identification of peptide epitopes and the design of experimental vaccines against MERS coronavirus. A recent study by Tahir Ul Qamar et al[96] identified both T-cell and B-cell epitopes from the MERS coronavirus S protein that were conserved across clinical isolates, suggesting that these epitopes may be used to develop broadly protective vaccines. A similar study focused on the MERS coronavirus N protein as a potential vaccine target, identifying candidate B-cell (15 linear, 10 conformational) and T-cell (10 helper T cell, 10 cytotoxic) epitopes for further

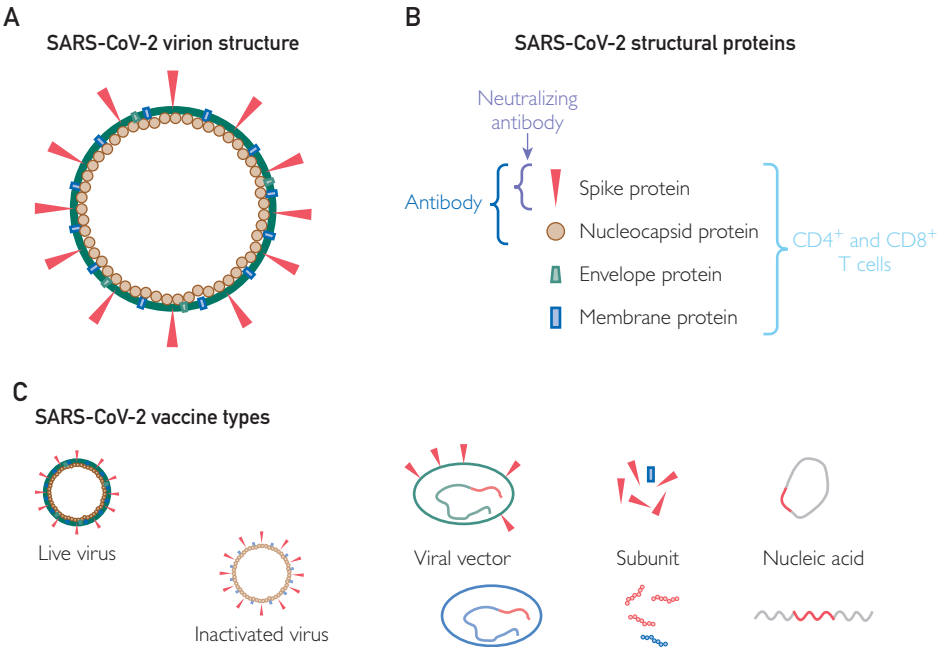

FIGURE. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines. A, Diagram of the SARS-CoV-2 virion, including the lipid membrane and structural proteins. B, The 4 major structural proteins are targeted by immune response. Humoral responses are directed at both the spike protein and the nucleocapsid proteins. Neutralizing antibodies have been identified that target the receptor-binding domain of the spike protein. All of the structural (and many of the nonstructural) proteins have predicted T-cell epitopes within them, suggesting that the T-cell response is likely able to recognize most viral proteins. C, Representation of the major types of SARS-CoV-2 vaccines under development. Live-virus vaccines typically consist of a weakened version of the virus, while whole inactivated vaccines use chemicals or radiation to eliminate viral replication. Vector-based vaccines incorporate one or more viral genes (in red) into the genome of a viral vector. Some vectors are replicating (eg, measles), while others may be replication-defective but are capable of limited transcription and expression of the desired coronavirus antigen. Subunit vaccines typically consist of specific viral proteins or immunogenic peptides derived from those proteins. Nucleic acid vaccines contain DNA (top figure) or RNA (bottom figure) that are delivered using electroporation or liposomal delivery systems that enable the nucleic acid to enter target cells. Viral protein is then produced by the host cells.

study.[97] Once individual peptide epitopes have been identified by immunoinformatic approaches, they can be computationally modeled as larger polypeptide assemblies for immunologic evaluation. Srivastava et al[98] employed such an approach to identify cytotoxic and helper T-cell epitopes from the MERS coronavirus proteome and design 2 vaccine constructs, both of which were predicted to provide broad global HLA population coverage (94%) and dock with TLRs. Applying similar immunoinformatic approaches, we comprehensively analyzed 10 SARS-CoV-2 proteins to identify potential targets for inclusion in COVID-19 vaccines.[99]

Similar studies detailing the *in silico* prediction of T-cell and B-cell epitopes from SARS-CoV-2 began to rapidly emerge following publication of the viral genome sequence. Grifoni et al[100] reported the bioinformatic identification of T-cell and B-cell epitopes from SARS-CoV-2 structural proteins that possessed high homology with immunogenic epitopes from SARS-CoV-1. A number of other studies have identified SARS-CoV-2 T-cell and B-cell epitopes a priori based on B-cell antigenicity scoring or

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TABLE 2. Clinical Trials Involving SARS-CoV-2 Vaccines

NCT number	Vaccine type	Sponsor/collaborators	Trial phase	Location
NCT04299724	Artificial APCs expressing SARS-CoV-2 proteins	Shenzhen Geno-Immune Medical Institute	I	Guangdong, China
NCT04383574	Alum-adjuvanted, formalin-inactivated vaccine	Sinovac Research and Development Co, Ltd	I/2	Hebei, China
NCT04352608	Alum-adjuvanted, formalin-inactivated vaccine	Sinovac Research and Development Co, Ltd	I/2	Jiangsu, China
NCT04450004	Virus-like particle vaccine	Medicago Inc	I	Not provided
NCT04412538	Inactivated SARS-CoV-2 vaccine	Chinese Academy of Medical Sciences, West China Second University Hospital, Yunnan Center for Disease Control and Prevention	I/2	Sichuan, China
NCT04283461	RNA vaccine: mRNA-1273	National Institute of Allergy and Infectious Diseases	I	United States
NCT04405908	Subunit vaccine: spike protein trimer	Clover Biopharmaceuticals AUS Pty Ltd	I	Australia
NCT04313127	Vectored vaccine: adenovirus type 5 vector	CanSino Biologics Inc, Institute of Biotechnology, Academy of Military Medical Sciences, PLA of China, Jiangsu Provincial Center for Disease Control and Prevention, Hubei Provincial Center for Disease Control and Prevention, Tongji Hospital	I	Hubei, China
NCT04437875	Vectored vaccine: adenovirus type 26 with spike protein	Gamaleya Research Institute of Epidemiology and Microbiology, Ministry of Health of the Russian Federation, Acellena Contract Drug Research and Development	I/2	Russia
NCT04368728	RNA vaccines: BNT162a1, BNT162b1, BNT162b2, BNT162c2	BioNTech SE, Pfizer Inc	I/2	United States
NCT04341389	Vectored vaccine: adenovirus type 5 vector	Insitute of Biotechnology, Academy of Military Medical Sciences, PLA of China, CanSino Biologics Inc, Jiangsu Provincial Center for Disease Control and Prevention, Hubei Provincial Center for Disease Control and Prevention, Zhongnan Hospital	2	Hubei, China
NCT04386252	Artificial APCs expressing SARS-CoV-2 proteins	AIVITA Biomedical, Inc	I/2	United States
NCT04368988	Nanoparticle vaccine with Matrix-M adjuvant	Novavax, Inc	I	Australia
NCT04324606	Vectored vaccine: chimpanzee adenovirus, ChAdOx1	University of Oxford	I/2	United Kingdom
NCT04334980	Oral vaccine: bacTRL-Spike	Symvivo Corporation	I	United States, Canada
NCT04405076	RNA vaccine: mRNA-1273	Moderna, Inc, Biomedical Advanced Research and Development Authority	2	United States
NCT04400838	Vectored vaccine: chimpanzee adenovirus, ChAdOx1	University of Oxford	2/3	United Kingdom
NCT04428073	Vectored vaccine: adeno-associated virus	GeneCure Biotechnologies	I	Not provided

Continued on next page

TABLE 2. Continued

NCT number	Vaccine type	Sponsor/collaborators	Trial phase	Location
NCT04453852	Recombinant protein vaccine with Advax-SM adjuvant	Vaxine Pty Ltd, Central Adelaide Local Health Network Incorporated	I	Australia
NCT04398147	Vectored vaccine: adenovirus type 5 vector	CanSino Biologics Inc, Beijing Institute of Biotechnology, Canadian Center for Vaccinology	I/2	Canada
NCT04444674	Vectored vaccine: chimpanzee adenovirus, ChAdOx1	University of Witwatersrand, South Africa, South African Medical Research Council, Bill and Melinda Gates Foundation, University of Oxford	I/2	South Africa
NCT04449276	Biological: CVnCoV vaccine; Drug: placebo	CureVac AG, Coalition for Epidemic Preparedness Innovations	I	Germany
NCT04447781	DNA vaccine: INO-4800	International Vaccine Institute, Coalition for Epidemic Preparedness Innovations, INOVIO Pharmaceuticals	I/2	Not provided
NCT04336410	DNA vaccine: INO-4800	INOVIO Pharmaceuticals, Coalition for Epidemic Preparedness Innovations	I	United States
NCT04380701	RNA vaccines: BNT162a1, BNT162b1, BNT162b2, BNT162c2	BioNTech RNA Pharmaceuticals GmbH, BioNTech SE	I/2	Germany

APCs = antigen-presenting cells; NCT = National Clinical Trial; PLA = People's Liberation Army; SARS-CoV-2 = severe acute respiratory syndrome coronavirus.

HLA binding affinity,[101-105] with several designing polypeptide vaccine candidates and modeling their binding with HLA and TLR molecules.[106-108] We have pursued a similar approach, stringently applying combinations of *in silico* approaches to identify subsets of T-cell ($CD4^+$ and $CD8^+$) and B-cell (linear and conformational) epitopes from the SARS-CoV-2 proteome to serve as candidates for peptide-based vaccine development.[99] These studies illustrate the utility of bioinformatics and computer-based predictive modeling for designing vaccines against rare and emerging diseases when immunologic data and biological samples are limited.

CURRENT STATUS OF VACCINE DEVELOPMENT

Some of the first vaccines are already in clinical trials 4 to 5 months after the start of the outbreak. As of the time of this writing, 1 vaccine has been licensed in China (only for use in the Chinese military), 3 vaccines are in phase 3 trials, 8 are in phase 2 trials, 11 are in phase 1 trials, and the remainder are in preclinical studies. This amazingly rapid development cycle is due to several factors: existing vaccine candidates, data, and animal models from SARS and MERS; the early publication of the full-length genome sequence of SARS-CoV-2; the striking sequence similarity in the S protein between SARS-CoV-1 and SARS-CoV-2; the use of DNA and RNA "plug and play" vaccine platforms; and reduced regulatory burdens due to the urgent nature of the outbreak (Figure).

Table 2 lists clinical trials currently under way. The first vaccine in clinical trials in the United States was the mRNA-1273 vaccine. This is a nonreplicating RNA vaccine that induces S protein production in host cells, leading to an antibody response. This vaccine was developed as a collaboration between the National Institutes of Health (NIH) Vaccine Research Center and Moderna, Inc. The clinical trial initially enrolled 45 adults aged 18 to 50 years who received an initial priming vaccine and a booster 4 weeks later. Jackson et al[109] reported a vaccine dose-dependent increase in serum antibodies to SARS-CoV-2 S2 and RBD regions of the S protein after the first

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dose and a significant boost on receipt of the second vaccination. Vaccinated recipients also developed antibodies capable of neutralizing both a pseudotyped lentivirus reporter and wild-type SARS-CoV-2. Examination of the T-cell responses in the 2 lower vaccine dose (25 μg and 100 μg) groups identified the presence of SARS-CoV-2–specific $CD4^+$ T cells with a Th1 phenotype. Virus-specific $CD8^+$ T cells were detected in the 100-μg vaccine group. With regard to safety, no serious adverse events were noted; however, fatigue, chills, headache, myalgia, and pain at the injection site were common (reported in >50% of recipients). Local adverse events were typically mild, although severity was more pronounced at higher doses. Of the 5 grade-2 adverse events noted, only 2 were deemed to be related to the vaccine. Both (elevated lipase and decreased hemoglobin) occurred 7 days after the second vaccination. The NIH is now recruiting 2 additional age groups (51 to 70 years and ≥71 years) to evaluate the vaccine in older populations in a phase 2 clinical trial and is seeking regulatory approval for a much larger-scale phase 3 trial that began during the summer of 2020. INOVIO Pharmaceuticals has developed a DNA-based vaccine that is injected and then electroporated into muscle cells in order to induce host cell production of the S protein.

There are over 120 additional vaccines in various stages of preclinical development, and the number increases weekly.[110-112] A wide variety of vaccine approaches are being used, including DNA and RNA vaccines, live coronavirus vaccines, inactivated virus vaccines, subunit vaccines (predominantly S protein), vectored vaccines (eg, vesicular stomatitis virus, adenovirus, MVA, measles virus), and peptide-based vaccines.

Live Virus Vaccines and Whole Inactivated Vaccines

Live virus and inactivated, whole-virus vaccine have an extensive history of success. They are the most immunogenic of the vaccine formulations; however, this comes at a price in terms of potential safety issues. Given the existing data that these vaccines can cause immunopathology and ADE, careful scrutiny of safety signals will be paramount during animal studies and clinical trials. Codagenix Inc and the Serum Institute of India are developing a live attenuated vaccine based on their CodaVax technology that uses codon-deoptimization to attenuate viruses. Influenza, respiratory syncytial virus, and DENV-2 vaccines based on this technology have documented both safety and immunogenicity in animal models.[113-115] The University of Hong Kong is developing an intranasal vaccine using an attenuated influenza virus (similar to what is in FluMist [AstraZeneca]) expressing the SARS-CoV2 S protein.

Subunit Vaccines

Subunit vaccines consist of viral proteins or protein fragments. The absence of infectious virus increases the safety profile and eliminates issues with viral inactivation or virulence reversion. The vast majority of the SARS-CoV-2 subunit vaccines have focused on the S protein or specific domains within the S protein, such as the RBD.[27] Other groups have focused on the N protein because studies with SARS-CoV-1 and MERS coronavirus have revealed that it is targeted by antibodies and contains HLA-restricted T-cell epitopes.[116,117] The proteins selected for use are often combined with adjuvants to boost immunogenicity. Large-scale production of the antigen can be problematic, although a variety of improved expression platforms, including plant-based systems, may provide high-throughput and scalable solutions.[118] Baylor College of Medicine is evaluating whether a SARS-CoV-1 recombinant protein vaccine provides protection against SARS-CoV-2. Novavax, Inc has received funding from the Coalition for Epidemic Preparedness Innovations to move its protein nanoparticle vaccine into clinical trials. This vaccine uses a saponin-based adjuvant, which is a formulation that has been found to enhance adaptive immune responses to recombinant Ebola virus glycoprotein vaccines and MVA-based influenza vaccines.[119,120] The Coalition for Epidemic Preparedness Innovations has also partnered with the University of Queensland

to develop a protein-based vaccine that uses a "molecular clamp" to lock the coronavirus proteins into the correct 3-dimensional shape, allowing humoral immune responses to develop against appropriate conformational epitopes.[121] Vaxart, Inc is developing an oral tablet-based vaccine that uses a replication-deficient adenovirus type 5 vector to deliver recombinant S protein and a TLR-3 adjuvant to the mucosal epithelium.[122] An Israeli company, MigVax Ltd, is also developing an oral subunit vaccine against COVID-19. This product is based on their existing vaccine against poultry coronaviruses[123] causing infectious bronchitis. The Mayo Clinic Vaccine Research Group is working on a peptide-based vaccine using naturally processed and presented epitopes from multiple SARS-CoV-2 proteins identified through mass spectrometry.[95,124-127] Virus-like particle (VLP) vaccines are a type of subunit vaccine consisting of an empty virus shell that lacks nucleic acid and is therefore noninfectious. The VLPs retain the 3-dimensional structure and repetitive antigenic nature of viral particles and have been found to be extremely immunogenic.[128] Nearly a dozen groups are working on VLP platforms expressing S protein or RBD. The University of Pittsburgh Medical Center has developed a microneedle skin patch vaccine for SARS-CoV-2 that induced neutralizing antibody production in mice.[129]

Nucleic Acid Vaccines

Nucleic acid vaccines can be rapidly and inexpensively produced and contain no live virus; however, DNA vaccines require complicated delivery systems and generally higher doses and are more difficult to produce. RNA vaccines may suffer from transfection efficiency issues in vivo. In addition to the INO-4800 and mRNA-1273 vaccines currently in clinical trials, Sanofi and the Biomedical Advanced Research and Development Authority are also working on a DNA vaccine. The NIH's Rocky Mountain Laboratories is also working with CureVac AG and with the University of Washington on additional RNA vaccine candidates. Tongji University in China has partnered with Stermirna Therapeutics Co, Ltd to develop an RNA-based vaccine.[130] The Imperial College London is developing a self-amplifying RNA vaccine. The Karolinska Institute and Cobra Biologics are also collaborating on a DNA vaccine.[131] Pfizer Inc and BioNTech SE have developed 4 mRNA-based formulations including 2 nucleoside-modified mRNAs, a uridine-containing mRNA, and a self-amplifying RNA. Results from a clinical trial of the BNT162b1 vaccine (encoding the RBD domain of the S protein) involving 45 participants aged 19 to 54 years was recently reported on *medRxiv*.[132] The authors indicate that the most common adverse effects were pain at the injection site, fatigue, and headache. The vaccine elicited RBD-binding antibody at similar titers to those seen in COVID-19—convalescent patients. The vaccine also elicited modest increases in SARS-CoV-2—neutralizing antibody titers. Studies evaluating the durability of the humoral response are ongoing.

Vectored Vaccines

Vector-based vaccines are a form of live attenuated vaccines that adapt existing successful and safe viral vectors (eg, vesicular stomatitis virus, adenovirus, MVA, measles) to express coronavirus proteins on immunization. Many of these vectors are not replication-competent in human cells, while others are only capable of limited replication and have defined safety profiles. Recombinant versions of their viral vectors can be rapidly produced, protein expression verified, and vaccines quickly developed. These platforms also have existing safety and immunogenicity data for other pathogens, which can further accelerate their development. CanSino Biologics Inc has multiple clinical trials investigating their adenovirus type 5 vectored vaccine. The initial phase 1 trial (NCT04313127) included 108 participants and tested 3 doses of the vaccine; the follow-up phase 1/2 trial (NCT04398147) includes 696 participants and is a randomized, observer-blind, dose-escalation trial in individuals 18 to 85 years of age. The third trial (phase 2: NCT04341389) includes 508 participants across 2 different doses of vaccine.

The NIH's Rocky Mountain Laboratories in Hamilton, Montana, is collaborating with the University of Oxford to develop and test a chimpanzee adenovirus (serotype Y25)—vectored SARS-CoV-2 vaccine.[133] Clinical trials, including a phase 1/2 trial (NCT04324606) involving 1090 participants and a phase 2/3 trial (NCT04400838) involving 10,260 participants are under way, as are additional trials in Brazil and South Africa (NCT04444674) aimed at studying the immunogenicity and efficacy in HIV-infected participants. Oxford University has partnered with AstraZeneca to produce hundreds of millions of vaccine doses.[134] The Biomedical Advanced Research and Development Authority and Janssen Research and Development, LLC have an adenovirus 26—vectored vaccine expressing the S protein. This vaccine is based on a platform that was used to rapidly create an investigational vaccine for Ebola virus. The Pasteur Institute, Themis Bioscience GmbH, and the University of Pittsburgh Center for Vaccine Research are developing a measles virus—vectored vaccine that expresses the SARS-CoV-2 S protein.[135] Investigators at Mayo Clinic have an adenovirus-vectored vaccine and a recombinant measles vaccine in preclinical development.

EXPERT COMMENTARY/LOOK AHEAD

Looking into the future, we see several issues relevant to COVID-19 vaccine development:

- Concerns over an "S-only" vaccine approach for an RNA virus and the possibility of viral mutation and recombination events[13] that could diminish or negate the efficacy of first generation vaccines
- Ongoing research into the optimal balance of vaccine-induced immunity (innate, humoral, cellular) is needed
- Discussion regarding controlled human challenge models and emergency use authorization approaches to development and use of candidate vaccines
- Concerns about antibody/vaccine—enhanced disease, as was observed in initial animal studies of SARS-CoV-1 vaccine candidates in both mice and ferrets
- The likely need for more than one vaccine type: those for immunoimmature (intranasal?); immunosenescent (adjuvanted or high dose?); immunocompromised (immunostimulant?); and pregnant (inactivated?) individuals
- The unknown efficacy and durability of vaccine-induced protection must be determined and inform vaccine administration regimens; will a "prime-boost" 2-dose strategy be needed? Periodic booster doses? Will vacinees need to be screened for preexisting antibody? Will annual boosters be needed?
- SARS-CoV-2 will very likely not be the last coronavirus to cause widespread and important human infections. Governments and funders must develop mechanisms for virus surveillance, as well as ongoing antiviral and vaccine development—even in the absence of current infections and beyond normal organizational attention spans
- A correlate of protection for immunity must be defined, whether for wild virus or vaccine-induced immunity
- Vaccine manufacturing and distribution capacity must be developed to provide ongoing immediate capacity for vaccine manufacture of new vaccines against novel human pathogens

CONCLUSION

SARS-CoV-2 is now circulating in both the Northern and Southern Hemispheres. Given the likelihood of severe disease due to risk factors, and less medical and public health infrastructure in the Southern Hemisphere compared with the Northern Hemisphere, the virus is likely to recirculate back to the Northern Hemisphere in the fall/winter of 2020-2021. For this reason, and due to the severity of the disease at the population level, a safe and efficacious vaccine against COVID-19 is imperative. While accelerated vaccine development must occur, it must do so without compromising safety when used in a variety of subpopulations. Much remains to be learned in regard to SARS-CoV-2 and vaccine development.

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Abbreviations and Acronyms: **ACE2** = angiotensin-converting enzyme 2; **ADE** = antibody-dependent enhancement; **COVID-19** = coronavirus disease 2019; **IL** = interleukin; **MERS** = Middle East respiratory syndrome; **MVA** = modified vaccinia virus Ankara; **NIH** = National Institutes of Health; **RBD** = receptor-binding domain; **S** = spike; **SARS** = severe acute respiratory syndrome; **SARS-CoV** = SARS coronavirus; **TLR** = Toll-like receptor; **VLP** = virus-like particle; **WHO** = World Health Organization

Potential Competing Interests: Dr Poland is the chair of a Safety Evaluation Committee for novel investigational vaccine trials being conducted by Merck Research Laboratories and is a consultant on vaccine development for Merck & Co, Inc, Avianax LLC, Adjuvance Technologies Inc, Valneva SE, Medicago Inc, GlaxoSmithKline plc, Sanofi Pasteur, Emergent BioSolutions Inc, Dynavax Technologies, Genentech, Inc, Eli Lilly and Company, Janssen Global Services, LLC, Kentucky BioProcessing, Inc, and Genevant Sciences Corporation. Drs Poland, Ovsyannikova, and Kennedy hold patents related to vaccinia, influenza, and measles peptide vaccines and have received grant funding from ICW Healthcare Ventures for preclinical studies on a peptide-based COVID-19 vaccine. Dr Kennedy has received funding from Merck Research Laboratories to study waning immunity to mumps vaccine. These activities have been reviewed by the Mayo Clinic Conflict of Interest Review Board and are conducted in compliance with Mayo Clinic Conflict of Interest policies. Dr Crooke reports no competing interests.

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