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REVIEW

Perspectives on development of vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)

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ABSTRACT

The recent outbreak of Coronavirus Disease 2019 (COVID-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has been characterized by the World Health Organization (WHO) as a controllable global pandemic. The spike (S) glycoprotein mediates binding to the angiotensin-converting enzyme 2 (ACE2) receptor for virus entry and also services as the target of virus-neutralizing antibodies, making it an attractive and leading viral antigen for vaccine development. No vaccine against any human coronavirus is available to date. In learning from the experience of developing Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV vaccine candidates in preclinical and clinical trials, the most promising strategies for SARS-CoV-2 vaccines should employ viral-vector platforms, properly adjuvanted recombinant protein or DNA/mRNA encoding an engineered sequence of trimeric S protein in pre-fusion conformation.

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The recent outbreak of Coronavirus Disease 2019 (COVID-19) has spread to essentially every country in the world. This prompted the WHO declaration of a public health emergency of international concern on Jan 30 and a global pandemic on March 11. The mortality rate of reported cases is approaching 7%. However, the actual number of infected is unknown and undoubtedly understated since no country has tested more than 50% of its residents. The retrospective investigation of 44,672 confirmed COVID-19 cases in Mainland China demonstrated that the overall case fatality rate was about 2.3% and more than half of cases were over 50-y old, who account for over 90% of deaths.[1] The common symptoms of COVID-19 are bilateral pneumonia, fever, cough, myalgia or fatigue.[2,3] Thus, this novel SARS-CoV-2 is more likely to result in severe and even fatal respiratory diseases in the elderly with comorbidities.

Thanks to the prompt sharing of the SARS-CoV-2 genome sequence by Chinese scientists, SARS-CoV-2 was identified as the seventh member of coronaviridae family that infects humans, including Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus (SARS-CoV). SARS-CoV-2 is a positive sense, single-stranded RNA virus with a genome of 29,891 nucleotides. Similar to other β coronaviruses, the SARS-CoV-2 genome contains two flanking untranslated regions (UTRs), a single long open reading frame (ORF) and 12 putative, functional ORFs.[4] The genomic characterization indicated that SARS-CoV-2 is closely related to the bat-derived SARS-like coronaviruses (Bat-SL-CoV).[5] Although the novel SARS-CoV-2 only shares approximately 79% of sequence identity to SARS-CoV, they use the same spike (S) glycoprotein to mediate the angiotensin-converting enzyme 2 (ACE2) receptor binding. In addition, the S protein of SARS-CoV-2 shows about 10- to 20-fold higher binding affinity to ACE2 than SARS-CoV.[5,6] The S glycoprotein is crucial for determining host tropism and the target of virus-neutralizing antibodies, making it an attractive and leading viral antigen candidate for vaccine development.

No vaccine against any human coronavirus is available to date, with protective efficacy in fighting against the SARS-CoV-2 pandemic. Since the epidemics of SARS-CoV in 2002 and MERS-CoV in 2012, dozens of vaccine candidates against coronaviruses have been evaluated in preclinical models and in early clinical studies, among them, the S glycoprotein was mostly used as vaccine antigen.[7] Vaccine candidates utilizing viral vector, subunit proteins and DNA platform based on the full-length S protein or receptor-binding domain (RBD) of SARS-CoV and MERS-CoV not only induced T-cell and neutralizing-antibody responses but also stimulated protective immunity in animal models.[8–17] Nevertheless, other viral antigens like nucleocapsid (N), membrane (M) and envelope (E) proteins failed to induce complete protective immunity against homologous SARS-CoV challenge in mice.[18,19] With two of SARS-CoV vaccines withdrawn, only five candidates based on S protein had advanced to early clinical trials, including four MERS-CoV and one SARS-CoV vaccine candidates (Table 1). The fact that two trials were withdrawn and only one phase I trial of SARS-CoV vaccine was completed might be due to the rapid disappearance of the virus. The lack of sufficient patients also hindered further clinical trials of MERS-CoV vaccines, while camels become another option to test efficacy of the vaccine 'ChAdOx1 MERS'.[20] It is noteworthy that three of these promising MERS/SARS vaccines are viral vector-based vaccines, and the other two are DNA vaccines. The phase I trial results of GLS-5300 and VRC-SRSDNA015-00-VP indicated that the vaccines were well tolerated and able to induce cellular immune responses and neutralizing antibody in about 80% of healthy adults.[21,22]

To date, over 120 enterprises and research institutes have claimed in public reports to initiate projects of SARS-CoV-2

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Table 1. Vaccine candidates of SARS-CoV and MERS-CoV in clinical trials.

Rank	Condition	Vaccine	Strategy	Antigen	Sponsor	Clinical Phase	Enrollment	Start Date	Status
1	MERS	BVRS-GamVac[10]	Human Ad-vector	Spike Protein	Health Ministry of the Russian Federation	I/II: NCT04128059 I/II: NCT04130594	268 (Estimated) 162 (Estimated)	Nov-19 Nov-19	Recruiting Recruiting
2	MERS	ChAdOx1 MERS[8,9,20]	Chimpanzee Ad-vector	Spike Protein	KAIMRC University of Oxford	I: NCT04170829 I: NCT03399578	24 (Estimated) 48 (Estimated)	Jan-20 Mar-18	Recruiting Recruiting
3	MERS	MVA-MERS-S[12,13]	MVA-vector	Spike Protein	UKE	I: NCT04119440	160 (Estimated)	May-20 (Estimated)	Not yet recruiting
					Marylyn Addo	I: NCT03615911	26	Nov-17	Completed
4	MERS	GLS-5300[16,21]	DNA Vaccine	Spike Protein	GeneOne Life Science, Inc.	I/II: NCT03721718 I: NCT02670187	60 75	Aug-18 Feb-16	Active Completed
5	SARS	D-3252	Subunit Vaccine	Spike Protein	NIAID	I: NCT01376765	0	Jun-11	Withdrawn
6	SARS	DIV	Inactivated Virus	Whole Virus	NIAID	I: NCT00533741	0	Sep-07	Withdrawn
7	SARS	VRC-SRSDNA015-00-VP[17,22]	DNA Vaccine	Spike Protein	NIAID	I: NCT00099463	10	Dec-04	Completed

Rank: the rank-order numbering is based on the starting date of each clinical trial. NCT numbers are those used in *clincialtrials.gov*. MERS: Middle East respiratory syndrome; SARS: severe acute respiratory syndrome; Ad: adenovirus; ChAdOx1: chimpanzee adenovirus Oxford 1; KAIMRC: King Abdullah International Medical Research Center. MVA: modified vaccinia Ankara; UKE: Universitätsklinikum Hamburg-Eppendorf. NIAID: National Institute of Allergy and Infectious Diseases. DIV: doubly inactivated SARS-CoV by treatment with formalin and ultraviolet light.

vaccine development. Among available new vaccine development technologies, the viral vector and DNA/mRNA platforms are the front-runners in terms of speed to enter clinical trials, having great potential for the vaccine development against this coronavirus pandemic. These platforms have many advantages including low cost, record of safety, allowing rapid vaccine design and construction for different pathogens. The manufacturing processes of these platforms have been demonstrated to be scalable (specially for DNA vaccine) and can meet the need of a quick response to the coronavirus pandemic. The mRNA-based mRNA-1273 and DNA-based INO-4800 COVID-19 vaccine candidates have entered phase I clinical trials in March and April, 2020 (ClinicalTrials.gov Identifier: NCT04283461, NCT04336410). Additionally, these vaccines are capable of inducing not only potent humoral immune responses but also balanced cellular immune responses. However, as a fact, no single candidate of DNA/mRNA vaccine has been approved yet, which might reflect the immature status of the technologies as compared with traditional approaches. Due to the relatively poor immunogenicity induced by naked DNA, DNA vaccines would need a specific, powerful electroporation (EP) device to achieve the desired effectiveness. So far, only limited immunogenicity and safety data of mRNA vaccines are available from clinical trials, and obtaining an optimal formulation for mRNA delivery is still one of the key challenges for human vaccine development.

The approvals of Ebola vaccines based on vesicular stomatitis virus (VSV-EBOV) and human adenovirus serotype 5 (Ad5-EBOV) demonstrate that recombinant viral vectors could be the most promising platform in the SARS-CoV-2 vaccine development and clinical applications.[23,24] A single injection of viral vector-based vaccines has been shown to be sufficient to induce high levels and long-term immune responses. An adenovirus (Ad5) vectored COVID-19 candidate vaccine has entered phase I clinical trial in March, 2020 (NCT04313127), and is expected to initiate phase II soon (NCT04341389). However, the preexisting immunity in human to the viral vector may dampen the vaccine efficacy; thus, a high dose of the vaccine has to be used in clinics.[25] In the clinical trial of Ad5-EBOV vaccine, the presence of high preexisting adenovirus vector neutralizing antibodies not only weakened the vaccine-elicited antibody responses but also accelerated the decline of antibody titers.[24] Alternatively, rare human serotypes or chimpanzee derived adenoviruses have been used for vaccine development with numerous safety data available in clinical trials. The recombinant replication-defective chimpanzee adenoviruses share the desirable features with human adenovirus such as the broad tissue tropism, the ease of large-scale manufacturing, a high foreign gene loading capacity that could easily accommodate the 3,822 bp of SARS-CoV-2 S coding sequence and could be prepared as freeze dried form that facilitates vaccine shelf life and transportation. Furthermore, adenoviral vector-based vaccines have the potential to stimulate mucosal immunity through oral or nasal vaccination, which was thought to be crucial in limiting SARS-CoV replication in the lungs of mice.[26,27]

Since the whole virus-based attenuated live or inactivated SARS-CoV-2 vaccine requires a BSL-3 GMP facility, recombinant S protein-based subunit vaccines could be an excellent alternative that is not limited by capacity for delivering a large number of vaccine doses. The cryo-EM structure showed that the trimer conformation of the SARS-CoV-2 S protein is much like MERS-CoV and that the prefusion conformation of MERS-CoV S protein is associated with a high titer of neutralization antibodies.[6,28] The results of a SARS-CoV DNA vaccine in mice indicated that the transmembrane domain of S protein, which gives rise to a more native trimer structure, could induce desired immunogenicity.[17] The RBD of S protein could also induce highly potent antibody responses but might be insufficient in inducing high levels of cellular immune response.[10] Additional concern with RBD-based vaccine is the possible immune evasion due to mutations; fortunately, recent sequence analysis of over 1,000 clinical isolates in China by the WHO team did not reveal

significant mutations. Another important aspect of the S protein-based vaccine development is the vaccine adjuvant selection. The vaccines composed of the same recombinant S protein of SARS-CoV with different adjuvants induced not only different levels of antibody responses but also different profiles of T-cell immune responses. Although the formulation with either Advax-1 (delta inulin) or Advax-2 (delta inulin + CpG 2006) adjuvants enhanced S protein-specific total IgG antibody responses significantly, the addition of CpG 2006 resulted in a significant increase in IgG2a and IFN-γ responses, but not IgG1 and IL-4, indicating a significant Th1 immune response. Further, the results of SARS-CoV virus challenge study indicated that vaccine-induced Th2-biased response was associated with the vaccine enhanced immunopathology with eosinophilic infiltrates within the lungs of challenged mice.[29] Similar associations with possible vaccine enhanced diseases were also reported in mice vaccinated by inactivated SARS vaccine alone or adjuvanted with alum or MF59.[30–32] Vaccine formulations with novel adjuvants, particularly the Toll-like receptor (TLR) ligands, including Poly(I:C) (TLR3), AS04 (TLR4), Imiquimod (TLR7/8) and CpG (TLR9), significantly triggers Th1 responses in animal studies.[33] Nevertheless, the comprehensive role of T-cell immunity in SARS-CoV-2 infection for vaccine development is still under investigation.

In summary, the ongoing pandemic over 200 countries will accelerate the development of SARS-CoV-2 vaccines. But the vaccines may help little in controlling the pandemic due to the long development time. Based on the experience of MERS-CoV and SARS-CoV vaccine development, the most promising strategies for a fast SARS-CoV-2 vaccine development should be employing technology platforms such as VSV/Ad-vector platforms, properly adjuvanted recombinant protein (superior in scale) or DNA/mRNA (superior in speed) encoding an engineered sequence of trimeric S protein in pre-fusion conformation.

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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