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Review

New vaccine production platforms used in developing SARS-CoV-2 vaccine candidates

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ABSTRACT

The threat of the current coronavirus disease pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is accelerating the development of potential vaccines. Candidate vaccines have been generated using existing technologies that have been applied for developing vaccines against other infectious diseases. Two new types of platforms, mRNA- and viral vector-based vaccines, have been gaining attention owing to the rapid advancement in their methodologies. In clinical trials, setting appropriate immunological endpoints plays a key role in evaluating the efficacy and safety of candidate vaccines. Updated information about immunological features from individuals who have or have not been exposed to SARS-CoV-2 continues to guide effective vaccine development strategies. This review highlights key strategies for generating candidate SARS-CoV-2 vaccines and considerations for vaccine development and clinical trials.

Contents

1. Features of SARS-CoV-2

Coronaviruses belong to the family of Coronaviridae (https://talk.ictvonline.org/). They are divided into four classes: alphacoronavirus and beta-coronavirus which infect mammals, and gamma-coronavirus and delta-coronavirus which primarily infect birds [1]. Currently, seven types of coronaviruses have been identified as infectious in humans (https://www.cdc.gov/coronavirus/types.html). Four of these types (HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1) have been defined as common human coronaviruses and have infected individuals around the world. The other three types (MERS-CoV, SARS-CoV, and SARS-CoV-2) cause acute respiratory diseases known as MERS, SARS, and COVID-19, respectively. Due to the circulation of common coronaviruses in the human population, pre-existing SARS-CoV-2-reactive T cells are observed in 40%–60% of unexposed individuals [2].

Coronaviruses have a large (~30 kb), single-stranded positive-sense RNA genome encoding several open reading frames [3]. Virus structures consist of spike proteins (S), membrane glycoproteins (M), nucleocapsid proteins (N), hemagglutinin-esterase dimer proteins (HE), and envelope proteins (E). S protein is a class I virus fusion protein that mediates attachment of the virus to cell surface

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receptors, which is then followed by uptake into endosomes [4–7]. Proteolytic cleavage of the S protein and fusion of viral and endosomal membranes trigger the release of viral RNA into the cytosol [8]. Cell entry of SARS-CoV-2 virus depends on binding of the viral S protein to the ACE2 receptor and the priming of S protein by serine protease TMPRSS2 [9,10]. The S proteins of SARS-CoV-2 and SARS-CoV have an amino acid sequence similarity of ~ 77% and can bind the same ACE2 receptor and cell protease TMPRSS2 [9,11].

Rapid bioinformatics techniques have been used to analyze the mutation dynamics of SARS-CoV-2, revealing how the virus has spread. The mutation rate of RNA viruses, such as influenza virus and HIV-1, is higher than that of DNA viruses [12]. However, genetic diversity analysis of the SARS-CoV-2 genome shows a notably lower rate of mutation than that in other RNA viruses. Regarding the S protein genome of SARS-CoV-2, a hotspot mutation was identified only at position D614 [13]. This is promising for vaccine development, which promotes vaccine-induced immunity targeting the receptor-binding domain of the S protein.

2. Candidate SARS-CoV-2 vaccines

Currently, there are over 100 candidate SARS-CoV-2 vaccines under development. The WHO is publishing a regularly updated list of vaccines in development (https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines). Platforms used to generate candidate vaccines are summarized in Table 1. Most candidate vaccines target surface membrane S protein, which is involved in receptor binding, membrane fusion, and entry into host cells.

Inactivated vaccines are a traditional method of manufacturing vaccines that are purified from virally infected cells [14]. Large-scale manufacturing methods established in the 1940s used embryonated eggs from hens to generate inactivated influenza vaccines [15]. Since then, many genetic engineering-based vaccine platforms have been developed to improve vaccine production [16]. Cell culture-based manufacturing technologies were developed in 2001 [14]. Since the manufacturing process is well established, inactivated SARS-CoV-2 vaccines have been developed rapidly, and six candidate vaccines are in clinical trials. A common technical issue for producing inactivated vaccines is the selection of suitable virus strains. An inactivated candidate vaccine, termed CoronaVac, derived from the CN2 strain with alum adjuvant showed broad neutralization ability against SARS-CoV-2 in preclinical studies [17]. At present, three of the inactivated vaccines are currently in phase III clinical trials (Table 1).

Compared to other vaccine platforms, the mRNA-based SARS-CoV-2 vaccine is more attractive because of its rapid and low-cost manufacturing process. Two mRNA candidate vaccines, mRNA-1273 and BNT162b1, are already in phase III clinical trials (Table 1). The mRNA-1273-encoded prefusion stabilizes the S protein, consisting of the SARS-CoV-2 glycoprotein with a transmembrane anchor and an intact S1–S2 cleavage site. This mRNA-1273 vaccine induced both humoral and cellular immunity in animal models and induced anti–SARS-CoV-2 immune responses in phase I clinical trials. Two immunizations of mRNA-1273 showed a well-tolerated safety profile and dose-dependent mild-to-moderate adverse events after the second immunization [18–21].

These mRNA-based vaccine platforms have been used to develop vaccines against infectious diseases, such as respiratory syncytial virus (RSV), Zika virus, influenza virus, Ebola virus, and HIV [22–24]. mRNA-based vaccines have favorable safety profiles, and there is no risk of infection since the virus does not need to be handled during manufacturing. mRNA has no risk of insertional mutagenesis because it does not need to enter the cellular nucleus to express the antigen. Repeated immunization with mRNA-based vaccines revealed long-term safety in murine models [25]. However, mRNA-based vaccines sometimes have issues with stability and translational efficiency. Improvements in mRNA-based vaccines have therefore been investigated [26]. For example, converting optimal codons enables an increase in mRNA stability. This codon optimization tool decreases the degradation of mRNA-based vaccines and increases the expression of encoded antigens [27]. Modification of the mRNA cap with synthesized anti-reverse cap analogs (ARCAs) improves translation efficiency by ensuring proper orientation [28]. ARCA-capped mRNAs also prolong protein expression and half-life in cells [29]. Self-amplifying mRNA can potentially improve vaccine efficiency, since lower doses are needed [30]. While no mRNA-based vaccines have been licensed for commercial use, there is a chance that one will be approved for the first time as a SARS-CoV-2 vaccine.

Viral vector-based candidate SARS-CoV-2 vaccines are also under development. These vaccines induce robust immune responses and can increase both humoral and cellular immunity [31]. An adenovirus type 5 (Ad5) vector-based SARS-CoV-2 vaccine, which encodes the S protein as a transgene antigen, showed tolerance and immunogenicity in phase I and is now in phase III clinical trials [32]. The concept of viral vectors was introduced with recombinant DNA from the SV40 virus in 1972 [33]. The vaccinia virus was subsequently used as a transient gene expression vector in 1982 [34]. Adenovirus vectors are easily grown to high titers in cell lines, have high transduction efficiency, have high transgene expression, and possess a broad range of viral tropism. Replication-defective Ad5 vectors can be created by deleting E1A and E1B viral gene regions, which have been well studied for candidate HIV-1 vaccines [35–37]. However, an Ad5 vector-based HIV-1 vaccine candidate failed during clinical trials due to pre-existing immunity against the Ad5 vector itself [38]. Therefore, developers of viral-vector-based SARS-CoV-2 vaccines should consider pre-existing immunity to the vectors. A phase I trial of an Ad5-nCoV (CanSino Biological Inc.) confirmed the result of diminishing vaccine efficiency in individuals with high pre-existing Ad5 immunity [32]. To induce a high and persistent immune response against SARS-CoV-2, investigation of prime-boost strategies with heterologous viral vectors, such as vaccinia virus, VSV, and alternated Ad, may be promising [39–42]. Using alternative Ad vectors, such as Ad26, Ad35, and nonhuman Ad-derived vectors that have low seroprevalence in humans may circumvent anti-vector immunity [43–45]. S protein-expressing chimpanzee adenovirus-based vaccine (ChAdOx1 nCoV-19) [46,47] and an Ad26 vector-based vaccine (Ad26.COV2-S) have initiated phase III clinical trials [48]. In phase I/II trials, a homologous ChAdOx1-nCoV-19 prime-boost regimen was safe, and even a single dose vaccination could induce both humoral and cellular immune responses [47]. Recombinant vesicular stomatitis virus (VSV) is also an attractive vector for SARS-CoV-2 vaccine development [49,50]. VSV vector-based vaccines are well studied, particularly VSVΔG-ZEBOV-GP, a vaccine against Ebola virus that was tested in clinical trials with 20,000 participants and licensed by the FDA in 2019 [51,52]. VSV vectors have low viral pathogenicity and rarely have pre-existing anti-vector immunity in humans. A live-attenuated, replication-competent, viral vector-based vaccine was developed where the VSV G gene was replaced with the S gene of SARS-CoV-2 (VSV-ΔG-spike). A single-dose vaccination of VSV-ΔG-spike was able to protect against SARS-CoV-2 in an animal model [53].

Plasmid DNA vaccine platforms have also been used to design SARS-CoV-2 candidate vaccines that have entered phase I/II clinical trials (Table 1). Similar to mRNA-based vaccines, DNA-based vaccines have low translational efficiency and weak immunogenicity compared to viral vector-based vaccines [54,55]. INO-4800 expresses S protein as an antigen using a novel electroporation medical device and showed protective immunity in animal models [56].

Table 1
Overview of technological platforms in candidate vaccines against SARS-CoV-2.

Platform	Advantage	Vaccine type	Vaccine name	Developers	Stage	Reference
Inactivated	Well established manufacturing process	Purified whole SARS-CoV-2 components	CoronaVac (PiCoVacc)	Sinovac	Phase III	[17]
			New Crown COVID-19	Wuhan Institute of Biological Products/ Sinopharm	Phase III	[80]
			BBIBP-CorV	Beijing Institute of Biological Products/ Sinopharm	Phase III	[81]
Nucleic acid	Rapid and low cost manufacturing	Lipid-encapsulated mRNA	mRNA1273	Moderna / NIAID	Phase III	[18–21]
			BNT162b1	BioNTech/Fosun Pharma/Pfizer	Phase III	[62,82]
		Self-amplifying mRNA	LNP-nCoVsaRNA	Imperial College London	Phase I	[30]
		Plasmid DNA with medical device	INO-4800	Inovio Pharmaceuticals / International Vaccine Institute	Phase I/II	[56]
Viral vector	Robust cellular and humoral vaccine immunity	Human adenovirus type5 (Ad5)	Ad5-nCoV	CanSino Biological Inc. / Beijing Institute of Biotechnology	Phase III	[32]
		Chimpanzee adenovirus (ChAd)	ChAdOx1 nCoV-19	AstraZeneca /University of Oxford	Phase III	[46,47]
		Human adenovirus type 26 (Ad26)	Ad26.COV2-S	Janssen Pharmaceutical companies	Phase III	[48,63,83]
		Vesicular stomatitis virus (VSV)	–	Merck / IAVI	Pre-clinical	[53]

3. Vaccine efficacy and safety in clinical trials

Immunological features that have been reported in individuals infected with SARS-CoV-2 have been useful for guiding vaccine development [4,57–59]. In COVID-19 patients given convalescent plasma from recovered patients, the immune response to S protein was robust and SARS-CoV-2-specific $CD8^+$ T cell responses to structural antigens, such as M and N, were also detected. Furthermore, SARS-CoV-2-specific $CD4^+$ T cell reactivity was observed in 40%–60% of unexposed individuals [2].

Classical vaccine development is based on adaptive defense mechanisms against viral infections. This information is critical for evaluating vaccine efficacy based on the immunological endpoints outlined for that particular vaccine. For example, if the purpose of a vaccine is to prevent absolute SARS-CoV-2 infection by robust induction of antiviral immunity, clinical trials should have appropriate endpoints based on the magnitude of humoral and/or cellular immunity. Appropriate endpoints do not only mean higher induction of antiviral protective immunity in certain human populations but should also consider the conversion rate of vaccinated populations. Since some vaccine efficacy is weaker in certain populations known as "low-responders" or "non-responders", the conversion rate of vaccinated populations provides useful information to estimate the variety of vaccine responses [60]. Although the magnitude of immune response sufficient to protect against SARS-CoV-2 infection remains unknown, immunogenicity is a key factor for vaccine development. In order to enhance vaccine immunogenicity, the number of doses, dosage amount, and time intervals need to be optimized. Subunit and peptide vaccines, in particular, have weak immunogenicity; therefore, these types of SARS-CoV-2 vaccines are often developed with novel adjuvant and delivery systems.

Second, if a vaccine is aimed at preventing infection from variants of SARS-CoV-2, clinical trials should have appropriate endpoints to evaluate cross-reactivity. Due to mutations in the viral genome, SARS-CoV-2 retains the potential to evade vaccine-induced antiviral immune responses. Some licensed vaccines (e.g., influenza) evaluate only neutralizing antibody production, and $CD4^+$ and $CD8^+$ T cell responses are not considered [61]. Some candidate vaccines have assessed antibody production as well as cellular T cell responses in their clinical trials [18–20,47,62,63]. $CD8^+$ T cell-mediated cellular immunity provides different antiviral responses from the neutralizing antibody, which plays a crucial role in viral clearance [64]. The protective role of pre-existing SARS-CoV-2 immunity is not yet clear, but vaccination may contribute to less severe symptoms upon SARS-CoV-2 infection. During the 2009 H1N1 influenza pandemic, pre-existing cross-reactive T cells in the adult population helped prevent severe disease [65]. It is difficult to evaluate the efficacy of vaccines focused on pathogenicity upon infection, but incorporating endpoints for T cell reactivity in response to SARS-CoV-2 proteins can demonstrate cross-reactivity of the vaccine. Viral genome sequencing identified fourteen mutation hotspot sites and predicted epitopes containing S genes [13]. Variants in S protein-encoding D614G increased infectivity in human lung epithelial cells. Although susceptibility to antisera neutralization may not be influenced, the D614G mutated variant has spread all over the world [66].

The tolerance and safety of candidate vaccines are evaluated in the early stages of clinical trials. In general, higher immunogenicity is associated with a higher frequency of adverse side effects, which is attributed to the use of a whole virion or adjuvant approach [67]. In some cases, virus-specific antibodies can enhance infection and replication, which is known as antibody-dependent enhancement (ADE) [68]. ADE depends on Fc receptors in host cells and contributes to clinical symptoms, such as acute respiratory injury, acute respiratory distress syndrome, and inflammation-based sequelae. Since ADE has been observed in the infection of dengue virus [69], Zika virus [70], Ebola virus [71], and coronaviruses [72,73] including SARS-CoV [74] and MERS-CoV [7568], the potential risk of vaccine-induced ADE should be evaluated in SARS-CoV-2 vaccine clinical trials.

Finally, clinical trial data regarding the persistence of vaccine-induced immune responses are important when evaluating effectiveness [76,77]. This will influence administration dosage and immunization schedules for the vaccine. Clinical features in COVID-19 patients suggest that asymptomatic individuals have a weaker immune response to SARS-CoV-2 infection and reduced neutralizing antibody levels within a few months [78,79]. During the pandemic, long-lasting protective immunity by single and low-dose administration will contribute to a prolonged availability of vaccine supply.

4. Conclusion

Each approach to developing SARS-CoV-2 vaccines has been challenging. Scientists are collaborating on a global scale and shar-

ing information and data promptly. During the pandemic, authorities are approving candidate vaccines faster and shortening licensing processes, which can normally take several years. In harmony with this, pharmaceutical companies are conducting clinical trials faster, focusing on large-scale and cost-effective manufacturing. In the near future, some candidate vaccines will be licensed and evaluated for their efficacy and safety in the global market. The experiences of COVID-19 provide helpful clues to combat the threat of emerging infectious diseases.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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