MINI-REVIEW

Highlight of severe acute respiratory syndrome coronavirus-2 vaccine development against COVID-19 pandemic

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Abstract: The pandemic caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has brought an unprecedented impact upon the global economy and public health. Although the SARS-CoV-2 virology has been gradually investigated, measures to combat this new threat in public health are still absent. To date, no certificated drug or vaccine has been developed for the treatment or prevention of coronavirus disease Extensive researches and international coordination has been conducted to rapidly develop novel vaccines against SARS-CoV-2 pandemic. Several major breakthroughs have been made through the identification of the genetic sequence and structural/non-structural proteins of SARS-CoV-2, which enabled the development of RNA-, DNA-based vaccines, subunit vaccines, and attenuated viral vaccines. In this review article, we present an overview of the recent advances of SARS-CoV-2 vaccines and the challenges that may be encountered in the development process, highlighting the advantages and disadvantages of these approaches that may help in effectively countering COVID-19.

Keywords: COVID-19; SARS-CoV-2; Vaccine development

1. INTRODUCTION

In December 2019, a sudden outbreak of a novel coronavirus (2019-nCoV, later named SARS-CoV-2) occurred in Wuhan, China, and spread rapidly around the globe. This virulent coronavirus caused majorly respiratory diseases, which affected not only the global healthcare system but also the world economy.[1,2] To date, the number of patients infected with SARS-CoV-2 has reached 31 million in more than 213 countries or territories, resulting in more than 1 205 437 deaths (up to November 2, 2020) (https://www.worldometers.info/coronavirus/). This catastrophic global pandemic necessitated the urgent development of precision diagnostic assays, valid treatment, and effective vaccines to combat escalating cases.[3] So Far, according to the World Health Organization's International Clinical Trials Registry Platform, more than 2200 clinical trials regarding prophylactic and therapeutic approaches are being performed in medical centers around the world.

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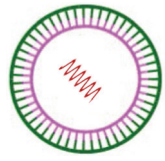
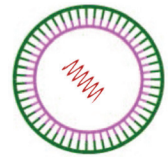
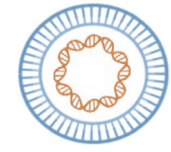
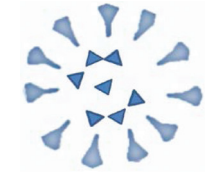
Remdesivir, a board-spectrum antiviral drug, was developed for treating Ebola virus disease and has been considered as an effective and safe treatment candidate for COVID-19.[4] Once Remdesivir enters the cell, it will be phosphorylated into remdesivir-TP and become a substrate for viral RNA-dependent RNA polymerase, resulting in a termination of the viral RNA synthesis.[5] Favipiravir (T-705), another prodrug of a purine nucleotide, has shown a 40% faster achievement of clinical improvement in a phase III trial conducted by Glenmark Pharmaceuticals (Mumbai, India).[6,7] Dexamethasone has been well-known as an anti-inflammatory and immunosuppressant steroid drug for the treatment of lung injury.[8–10] Preliminary results provided strong evidence indicating that dexamethasone increased the survival rate of 19 SARS-CoV2-infected patients, comparing with those who received usual care (29.3% vs 41.4%; rate ratio, 0.64).[11] Although dexamethasone and other corticosteroids are only used in severe patients who needed respiratory support, so far they remain the promising drugs for treating COVID-19–induced respiratory manifestations. Despite the efficacy of corticosteroids, vaccine development appears to be the only potential method to stop the spread of COVID-19. Generally, the development of a new vaccine requires 10 to 15 years, but the SARS-CoV2–related research progressed quickly in past few months, leading to a major breakthrough in the development of novel vaccines. Genetic sequencing and identifying the structural/nonstructural proteins of SARS-CoV-2 have enabled the development of RNA, DNA, and subunit vaccines as well as attenuated viral vaccines that can activate the host immune responses against the viruses.[12] Here, we summarize the current status of several in-development vaccines (Table 1).

Table 1

SARS-CoV-2 vaccine candidates in clinical trials

Research group	Moderna/NIAID	BioNTech/Pfizer/ Fosun Pharma	AstraZeneca/ The University of Oxford	Medigen
Vaccine type	mRNA vaccine	mRNA vaccine	DNA vaccine	Subunit Vaccine
Product name	mRNA-1273	BNT162b1/b2	ChadOx1	MVC-COV1901
Clinicaltrials.gov	NCT04470427	NCT04368728	NCT04400838	NCT04487210
Current phase	Phase 3	Phase 2/3	Phase 2/3	Phase 1
Schematic diagram				

2. DNA VACCINE

A DNA vaccine comprises a plasmid DNA that carries genes components, mainly protein or glycoprotein, of a pathogen that is proficient in stimulating host immune responses.[13,14] Adjuncts or adjuvants are often accompanied by to assist DNA entry in specific cells, or stimulating proper immunogenic responses.[15–17] Several disease outbreaks in the past, including the 2003 SARS coronavirus, 2005 H5N1 avian influenza, 2009 H1N1 influenza pandemic, and 2016 Zika virus, had all been issued with DNA vaccines for disease control.[14,18–22] DNA vaccines have various advantages, like the relative ease and low cost of production, fast to produce and modify, and long-term persistence of immunogen, which making them suitable for production in the developing system and the ability to be boosted by subsequent immunizations. Although, DNA vaccines still face many challenges to be an effective tool, including inducing antibody production against DNA, may have relatively poor immunogenicity, and the risks of insertion of foreign DNA into the host genome that may cause the cell to become cancerous.[23] Based on these concerning issues, including the finer detail regarding the way of vaccination, the adjuvant, and the genetic structure of the vaccine, still need to further elucidate.[24–27] Previous studies showed that DNA vaccines encoded the influenza NP gene, influenza HA or M gene, and the HIV gag gene have been demonstrated with fewer risk for host genome in the mice and guinea pigs.[28,29] Recently, it has been reported that DNA vaccine encoding the SARS-CoV-2 S protein-elicited neutralizing antibody titers correlated with protective efficacy in rhesus macaques after challenge with SARS-CoV-2.[30] At present, there are many S protein-based DNA vaccines are undergoing preclinical and phase 1/2 clinical trials, like INO-4800 developed by Inovio Pharmaceuticals Inc. (Philadelphia, PA). Many companies also devote to the development of S protein-based DNA vaccines for COVID-19 now.[31] Thanks to the well-established manufacturing process of DNA vaccines, the development of vaccines against infectious diseases, such as SARS-CoV-2, can be put into practice within a short period of time.

Folegatti et al[32] performed a phase 1/2, observer-blinded clinical trial on 1077 healthy adults aging between 18 and 55 y/o with negative SARS-CoV-2 infection and examined their response to DNA vaccine candidate "ChAdOx1 nCoV-19" to verify its safety and immunogenicity response in human. ChAdOx1 is a chimpanzee adenoviral vector-based vaccine with deficient replication ability and known to be immunogenic in older and immunocompromised individuals.[33] In previous studies, ChAdOx1 MERS, another strain of the ChAdOx1 vaccine that encodes the structural surface glycoprotein (spike protein) of the Middle East respiratory syndrome (MERS) virus, has undergone phase 1 clinical trial and offered humoral and cellular response against MERS-CoV.[34,35] Similar to ChAdOx1 MERS, ChAdOx1 nCoV-19 encodes the spike protein of SARS-CoV-2 and has been proven to be protective against SARS-CoV infection in *rhesus macaques* and other non-human primates, thus making it promising for future development in humans. In the trial, participants were randomly assigned to receive 0.5 mL injection of ChAdOx1 nCoV-19 (n = 543) or meningococcal conjugate vaccine (MenACWY, n=534) as control.[32] Meningococcal conjugate vaccine is a vaccine generally inoculated in countries affected by the bacterium Neisseria meningitides, which could lead to meningococcal meningitis.[36] A group of 10 participants were enrolled in a 28-day interval nonrandomized two dose administration for further assessments. Participants were observed clinic for at least 30 minutes for safety concerns and were asked to record local and systemic adverse events for 28 days after each vaccination. Blood samples were collected at days 0 and 28 for analysis and were expected for a 1-year follow-up.[32]

In the one-dose ChAdOx1 nCoV-19 group, serum antibodies against SARS-CoV-2 spike protein peaked at day 28 and remained such level to day 56. Besides, virus neutralization ability was detected in more than 90% of participants, and more than 60% of participants' sera had detectable SARS-CoV-2 cytopathic effect inhibition ability at day 56. In the two-dose group, all receivers were tested positive for virus neutralization and inhibition abilities. Local and systemic events occurred most often on the first day of vaccination. Pain and tenderness were the most common local adverse events in both vaccine groups and were greatly reduced when participants received prophylactic paracetamol. Systemic adverse events including fatigue, headache, muscle ache, malaise, and chills were generally mild to moderate and also reduced after prophylactic paracetamol administration.[32] In spite of DNA vaccines offer many advantages over conventional vaccines, there are still many challenges in resources, manufacturing, the implementation of vaccination programs, different vaccine platforms and strategies around the globe, need to be overcome.[37]

3. RNA VACCINE

RNA vaccine consists of an mRNA strand that codes for a disease-specific antigen and requires translation in the host to be expressed.[38,39] Wolff et al[40] introduced the concept of it in 1990 by directly injecting mRNA into the muscle, which finally led to the expression of the encoded protein. RNA vaccines possess similar advantages as well as DNA vaccines, like noninfectious, natural degradation, rapid and scalable production, stimulation of innate immune response and induction of T- and B-cell

immune response.[41] But opposed to DNA vaccines, RNA vaccines do not need to cross the nuclear envelop and easily degrade in the cells, which greatly reduce the risk of integration into the host genome.[42] The RNA vaccine also has its disadvantages, like its instability and low immunogenicity, which may decrease potency via multiple pathways, and the concern about the optimal formulation for vaccination.[41–43] Although RNA vaccines also have been investigated for therapeutic vaccines in past two dacades,[41] there are still difficulties for the development strategy of RNA vaccines in RNA virus, like HIV with higher mutation rate, and may have to adapt combination immunogen design and finer delivery strategies to stimulate similar responses.[44,45] However, challenges that impede the successful translation of these molecules into drugs are that mRNA is intrinsically unstable and prone to degradation by nucleases, and too large to be delivered into cells.[46] Nonetheless, recent technological advances have largely overcome these issues. Here, we report two mRNA vaccine candidates developed respectively by Jackson et al and Walsh et al.[47,48]

A phase 1, dose-escalation, open-label clinical trial was conducted by Jackson et al.[47] to determine the safety and efficacy of the candidate vaccine mRNA-1273. mRNA-1273, encoding the perfusion stabilized S protein antigen (S-2P) and capsulated by four lipid nanoparticles, was provided at dose levels of 25, 100, or 250 μg to 45 healthy adults aging between 18 and 55 y/o (n = 15 for each group). Participants received two vaccine injections into the deltoid muscle 28 days apart and were asked to record local and systemic adverse events for 7 days after each vaccination. Safety and immunogenicity data were recorded in the interim report. Test results from participants' sera indicated that higher dosages of both vaccines induced more effective S-2P and receptor-binding domain-specific antibodies. SARS-CoV-2 antibody-neutralizing activity was identified in all participants after the second vaccine administration. 25-μg dose group had the lowest response (50% inhibitory dilution, ID50 = 112.3), while 100- and 250-μg dose groups had higher and similar responses (ID50 = 343.8 and 332.2, respectively). Mild or moderate systemic adverse events were reported after first vaccination (5 in 25-μg group, 10 in 100-μg group, and 8 in 250-μg group) and became more often after second vaccination (7 in 25-μg group, 15 in 100-μg group, and 14 in 250-μg group) with three participants experiencing severe events. With satisfying immunogenic response outcomes and no trial-halting safety rules met, further development of the vaccine candidate is being expected.[47]

Walsh et al. conducted another placebo-controlled, observer-blinded dose-escalation phase 1 trial on vaccine candidates "BNT162b1" and "BNT162b2" following previous clinical trial results on BNT162b1 in 18 to 55 y/o participants.[48–50] Both capsulated by lipid nanoparticles, BNT162b1 encodes trimerized SARS-CoV-2 receptor binding domain, while BNT162b2 encodes the entire SARS-CoV-2 spike with two proline substitutions. Healthy participants (n = 195) were randomly assigned to vaccine groups defined by vaccine candidate (BNT162b1or BNT162b2), dose level (10 μg, 20 μg, 30 μg, 40 μg, 100 μg, or placebo), and age range (18-55 y/o or 65-85 y/o). Participants were given two 0.5-mL vaccine injections into the deltoid 21 days apart and observed for safety concerns for 30 minutes each time.[48]

Vaccine candidates BNT162b1 and BNT162b2 were immunogenic, inducing antibody binding, and neutralizing responses after the second injection. Though immunogenicity of both vaccine candidates decreased in an age-dependent fashion, neutralizing geometric mean titer still exceeded that of the convalescent serum panel 7 days after second vaccine administration. Dose-dependent antibody neutralizing responses were observed between 10- and 20-μg groups but not between 20- and 30-μg groups. Local adverse events were overall mild or moderate, and pain at the injection site was common. Systemic adverse events including fatigue, fever, and chill occurred most often in a dose-dependent manner after BNT162b1 vaccination. Dose 2 triggered more and aggravating systemic adverse events than dose 1, with a 33% 65 to 85 y/o participants reporting fever ≥38°C. Participants injected with BNT162b2 experienced milder systemic adverse events compared with those with BNT162b1 injection. No grade 4 systemic adverse events (emergency room visit or hospitalization) were reported in both vaccine groups.[48]

4. SUBUNIT VACCINE

Rather than introducing the entire pathogen, subunit vaccines include purified proteins or glycoprotein from the pathogen of interest.[51] Efficient in inducing humoral- and cell-mediated immunological responses and the risks associated with handling the pathogen eliminated, subunit vaccines have distinct advantages over live attenuated and inactivated vaccines.[52] Although subunit vaccines are safer and easier to be produced, adjuvants are typically required to achieve optimal immunogenic responses and long-term immunity.[53]

A subunit vaccine candidate, under development by Kuo et al,[54] consists of spike protein, aluminum salt, and TLR agonist. The ectodomain spike protein used in the study is in S-2P form, generated by expressing a plasmid encoding SARS-CoV-2 S protein with S1/S2 furin-recognition site (682-RRAR-685 to GSAS) and S2 central helix (986-KV-987 to PP) mutations in ExpiCHO cells. S protein structure was later verified by Cryo-EM. Aluminum hydroxide in combination with CpG 1018 adjuvant has been characterized to elicit Th1-biased immune response while retaining high antibody levels and was thus chosen as the adjuvants in the study.[54]

Potential for further in-human clinical trial development of the vaccine was confirmed by administrating BABL/cJ mice with different dosages of S-2P protein, CpG 1018(TLR agonist), and aluminum hydroxide. Besides the ability to generate immunogenicity, neutralizing antibody level increased in a dose-dependent manner. Neutralization capability against wild-type SARS-CoV-2 and pseudovirus carrying wild-type D614 and mutant D614G spike proteins was tested positive in mouse sera. No adverse events were reported when SD rats were injected with S-2P and the above-mentioned adjuvants.[54]

In conclusion, the current pandemic of COVID-19 caused by SARS-CoV-2 rapidly global spreads, which is also to date with high mortality rate beyond control. There still lacks satisfactory vaccines given to the patients for recovery.[55] Although increasing vaccine candidates were qualified to enter the next phase of clinical trials, several challenges still existed. First, although neutralizing responses of candidate vaccines were detected, the degree of protection these candidate vaccines can provide against SARS-CoV2 infection is unclear. Second, the scale of time course was relatively small in these clinical trials. Whether these candidate vaccines can offer long-lasting protection remains uncertain and to be answers in the future. Third, the subjects enrolled in these studies were all healthy adults. The efficacy of these candidate vaccines will need to be verified in a more diversified population, for example, older and younger individuals, immunocompromised individuals, and those with ongoing chronic diseases. As described in this article, the candidate vaccine ChAdOx1 that has entered the next phase 2/3 clinical trial has been halted due to an adverse effect with unknown cause. The clinical trial will be resumed once no correlation is identified between the candidate vaccine and the adverse effect. In conclusion, these vaccine candidates hold promises in offering potentials solution for the COVID-19 pandemic. However, more clinical trials with a longer time course or using diversified populations are required to evaluate the feasibilities of candidate vaccines before launching them to the markets.

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