COVID-19 Vaccine: Critical Questions with Complicated Answers

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Abstract

COVID-19 has caused extensive human casualties with significant economic impacts around the globe, and has imposed new challenges on health systems worldwide. Over the past decade, SARS, Ebola, and Zika also led to significant concerns among the scientific community. Interestingly, the SARS and Zika epidemics ended before vaccine development; however, the scholarly community and the pharmaceutical companies responded very quickly at that time. Similarly, when the genetic sequence of SARS-CoV-2 was revealed, global vaccine companies and scientists have stepped forward to develop a vaccine, triggering a race toward vaccine development that the whole world is relying on. Similarly, an effective and safe vaccine could play a pivotal role in eradicating COVID-19. However, few important questions regarding SARS-CoV-2 vaccine development are explored in this review.

Key Words: COVID-19, Vaccine, Vaccine backfires, Vaccine safety

INTRODUCTION

Humans have a long history of battling against the viruses. The constant battle between viruses and scientists has been recognized as a key driver of medical advances. In this series of battles, the latest one is the outbreak of a novel coronavirus, COVID-19, originating in the Wuhan region of China in late December 2019 (Ratan et al., 2020). COVID-19 has affected at least 216 countries, areas or territories around the world. So far, there had been approximately 54 million 301 thousand 156 cases and one million 316 thousand 994 deaths confirmed globally due to this deadly virus by November 16, 2020 (World Health Organization, 2020). Prior to the emergence of Severe Acute Respiratory Syndrome CoV-2 (SARS-CoV-2), there were six human CoVs (HCoVs), including that which caused the SARS global outbreak that started in November 2002 in the Guangdong province of China. After that epidemic, China reported more than 8,000 cases of disease and 774 deaths with a case-fatality rate of 7%. A decade later in 2012, Middle East respiratory syndrome CoV (MERS-CoV) first emerged in Saudi Arabia, with a total of 2,494 laboratory-confirmed cases and 858 deaths with a case-fatality ratio of 34.4% (Corman et al., 2018; Peeri et al., 2020). However, COVID-19 has caused a pandemic that has compelled the global economy to grind to a halt. A vaccine remains the best option for restoring normal life and global economies. This has triggered a vaccine development race. According to Mullard’s report (Mullard, 2020), as of 11 November 2020, there were 259 COVID-19 vaccine projects going on around the world. Of those, there were 79 protein, 16 virus-like particle, 22 DNA, 33 RNA, 36 non-replicating and 20 replicating viral vector, 15 inactivated and four live-attenuated, and 35 other vaccines can-
Among them, 204 were still in the preclinical stage; 11 candidates were reached phase III clinical trial stage, and the remaining were in between these stages (Fig. 1) (London School of Hygiene and Tropical Medicine, 2020).

A vaccine introduces the structure and biological agents of a specific virus to antigen-presenting cells of the host, which engulf it and pass portions of it to activate helper T (Th) cells. The Th cells then trigger other immune responses i.e., activation of B cells and cytotoxic T (Tc) cells. B cells produce antibodies that can prevent the virus from infecting cells, while Tc cells recognize and kill cells that are infected with the virus, that help the surveillance cells of the body to track the virus for long periods (Fig. 2B). In principle, understanding the etiology, epidemiology, pathogenesis and immunobiology of the infection is of the utmost importance for the development of vaccines (Zepp, 2010). Thus, a few simple questions, although complicated to answer, have arisen regarding the basic principles of vaccines that need to be resolved with regard to COVID-19 vaccine development. Here, we aim to address those simple questions.

**STRUCTURE AND PATHOPHYSIOLOGY OF COVID-19**

SARS-CoV-2 is a β-coronavirus belonging to the Sarbecovirus subgenus of the Coronaviridae family, and is enveloped with non-segmented positive-sense RNA virus (Zhu et al., 2020). In broad terms, the genome of this virus can be divided into two parts. The first open reading frame (ORF 1a/b) comprises two-thirds of the total viral genome (~30 kb) and encodes 16 non-structure proteins. This ORF 1a/b has the genetic function to roll-out the viral replication that controls the production of cellular proteins and keeps evading the immune system of the host. The remaining portion of the genome codes for four basic structural proteins, including spike (S) glycoprotein, small envelope (E) protein, matrix (M) protein, and nucleocapsid (N) protein, and several other accessory proteins (Guo et al., 2020). The crucial part of SARS-CoV-19 and COVID-19 infection begins when the S protein of this pathogen binds to angiotensin-converting enzyme 2 (ACE2), a cellular receptor of the host (Fig. 2A). After binding to the ACE2 receptor, the conformation shift in the S protein enables fusion of the viral envelope with the cell membrane via the endosomal pathway. Then, the virus enters the cell and releases its RNA. This RNA then goes through transcription, translation, and replication. In this production line, RNA is translated into replicase polyproteins pp1a and 1ab, and those are then severed by viral proteinase into small products. Polymerase by discontinuous transcription yields a sequence of subgenomic mRNAs that are eventually transformed into specific viral proteins. Subsequently, in the endoplasmic reticulum and Golgi apparatus, viral proteins and genome RNA are packaged into the virion and then transported through vesicles to be released from the cell (Shereen et al., 2020).

**TYPES OF VACCINE**

The Coalition for Epidemic Preparedness Innovations (CEPI), a multilateral and multinational stakeholders foun-
dation for the development of vaccine against infectious diseases, informed in September 2020 that the nine separate technological platforms are being used to make an effective vaccine against SARS-CoV-2 (Gopinathan et al., 2020). Scientist around the world using both the classical and next-generation platforms. The classical platforms are whole-inactivated virus, live-attenuated virus, protein subunit, and virus-like particles, and the next-generation platforms are nucleic acids (RNA and DNA), viral vectors (non-replicating and replicating), recombinant protein, and antigen-presenting cells (Le et al., 2020; van Riel and de Wit, 2020). However, as of September 2020 reported by CEPI, most of the frontrunner vaccines in clinical studies have emphasized on spike protein of coronavirus and its versions as the key antigen responsible for the infection of COVID-19. CEPI also pointed out that eleven candidate vaccine in clinical stage using adjuvant to improve the immunogenicity (Le et al., 2020).

Among the ongoing or planned clinical trial as of 11 November 2020, double-blind, single-blind, dose-confirmation, observer-blind randomized, and open-label non-randomized studies are designed for different types of the vaccine from the various technological platform, and of them, there are 18 nucleic acids, 8 Non-replicating viral vectors, 2 replicating viral vector, 9 inactivated virus, 1 virus-like particle, 11 protein subunit, and 3 other candidate vaccines. Moreover, on this clinical trial tally, China is on the top followed by the USA, Australia, Canada, UK, and others (Table 1).

THE CRITICAL QUESTIONS

The challenge for modern vaccinology is to be able to provoke all the requisite steps leading to immune system activation in vivo, and to provide a non-virulent, harmless type of a given agent capable of generating a strong and adequate immune response tailored against specific viral attack (Moser and Leo, 2010). Thus, some questions arise regarding the development of the vaccine (see Table 1 for current development state of COVID-19 vaccines) that will be administered to billions of people at risk of COVID-19 infection.

WILL VACCINE STIMULATE THE IMMUNE RESPONSE?

As mentioned earlier, ACE2 is the route of SARS-CoV-2 infection. However, this receptor plays a vital role in both innate and adaptive immune responses by modulating the antigen present antigen cells that interact with T cells to initiate defense initiatives (Bernstein et al., 2018). This receptor of transmembrane protease acts in the conversion of angiotensin 1-8 (Ang II) to angiotensin 1-7 (Ang 1-7), prompting diuresis/natriuresis, preserving renal function, and attenuating cardiac and vascular reformation (Vickers et al., 2002; Santos et al., 2008; Zhang et al., 2010). ACE2 also has an important role in the nervous system, and disruption of this receptor can trigger neurological disorders (Kabbani and Olds, 2020). However, a study reported that innate T cells, a heterogeneous class of T lymphocytes (MAIT, γδT and iNKT cells), are also altered by SARS-CoV-2 (Jouan et al., 2020). Besides, a study cohort of 38 patients found that a decline in T cells, B cells, and NK cells was linked to SARS due to coronavirus (Cui et al., 2003). On the other hand, a study conducted on bronchoalveolar lavage fluid of eight COVID-19 patients exhibited

Fig. 2. COVID-19 and the vaccine. (A) A simple representation of the COVID-19 infection mechanism in the body. (B) Basic principles of vaccines in generalized form.
### Table 1. Ongoing or planned clinical trial for COVID-19 vaccine up to 11 November 2020

| Candidate               | Type                | Phase | Study design                           | Volunteer | Country                          | Reference        |
|-------------------------|---------------------|-------|----------------------------------------|-----------|----------------------------------|------------------|
| Moderna mRNA-1273       | RNA                 | 3     | Double-blind randomized                | 30,000    | USA                              | NCT04470427      |
| WIBP vaccine            | Inactivated         | 3     | Double-blind randomized                | 45,000    | Bahrain, Jordan, Egypt, UAE      | NCT04510207      |
| Sinovac CoronaVac       | Inactivated         | 3     | Double-blind randomized                | 13,060    | Brazil                           | NCT04456595      |
| Oxford ChAdOx1-S        | Non-replicating     | 3     | Double-blind randomized                | 40,051    | USA, Chile, Peru                  | NCT04516746      |
| Novavax NVX-CoV2373     | Protein subunit     | 3     | Double-blind randomized                | 30,000    | USA, Mexico, Puerto Rico         | NCT04611802      |
| Novavax NVX-CoV2373     | Protein subunit     | 3     | Double-blind randomized                | 9,000     | UK                               | NCT04583995      |
| Moderna mRNA-1273       | RNA                 | 3     | Double-blind randomized                | 30,000    | USA                              | NCT04470427      |
| Cansino Ad5-nCoV        | Non-replicating     | 3     | Double-blind randomized                | 40,000    | Pakistan                         | NCT04526990      |
| BIBP/ Sinopharm BBIBP-CorV | Inactivated       | 3     | Double-blind randomized                | 3,000     | Argentina                        | NCT04560881      |
| Oxford ChAdOx1-S        | Non-replicating     | 2/3   | Single-blind randomized                | 12,390    | UK                               | NCT04400838      |
| BioNTech BNT162         | RNA                 | 2/3   | Dose-finding, double-blind randomized  | 43,998    | USA, Argentina, Brazil, others   | NCT04368728      |
| BioNTech BNT162         | RNA                 | 2/3   | Dose-finding, open-label non-randomized| 43,998    | USA, Argentina, Brazil, others   | NCT04380701      |
| AZLB protein subunit    | Protein subunit     | 2     | Double-blind randomized                | 900       | China                            | NCT04466085      |
| Novavax NVX-CoV2373     | Protein subunit     | 2     | Single-blind randomized                | 4,400     | South Africa                     | NCT04533999      |
| Curevac CVnCoV          | RNA                 | 2     | Dose-confirmation, double-blind randomized | 691       | Peru                             | NCT04515147      |
| Oxford ChAdOx1-S        | Non-replicating     | 1/2   | Double-blind randomized                | 2000      | South Africa                     | NCT04444674      |
| WIBP vaccine            | Inactivated         | 1/2   | Dose-finding, double-blind randomized  | 1,264     | China                            | ChiCTR2000031809  |
| Bharat Covaxin         | Inactivated         | 1/2   | Double-blind randomized                | 755       | India                            | NCT04471519      |
| Oxford ChAdOx1-S        | Non-replicating     | 1/2   | Single-blind randomized                | 1,090     | UK                               | NCT04324606      |
| Zydus Cadila ZyCoV-D    | DNA                 | 1/2   | Double-blind randomized                | 1048      | India                            | CTRI/2020/07/026352 |
| CAMS vaccine            | Inactivated         | 1/2   | Dose-finding, double-blind randomized  | 942       | China                            | NCT04412538      |
| Sinovac CoronaVac       | Inactivated         | 1/2   | Dose-finding, double-blind randomized  | 744       | China                            | NCT04352608      |
| Cansino Ad5-nCoV        | Non-replicating     | 1/2   | Dose-finding, double-blind randomized  | 696       | Canada                           | NCT04398147      |
| CAMS vaccine            | Inactivated         | 1/2   | Double-blind randomized                | 471       | China                            | NCT04470609      |
| Sinovac CoronaVac       | Inactivated         | 1/2   | Dose-finding, double-blind randomized  | 422       | China                            | NCT04383574      |
| Genexine GX-19          | DNA                 | 1/2   | Dose-finding, double-blind randomized  | 210       | Republic of Korea                | NCT04445389      |
### Table 1. COVID-19 Vaccine and Important Questions

| Candidate          | Type                | Phase | Study design                          | Volunteer | Country                | Reference       |
|--------------------|---------------------|-------|---------------------------------------|-----------|------------------------|-----------------|
| Aivita AV-COVID-19 | Other               | 1/2   | Dose-finding, double-blind randomized | 180       | USA                    | NCT04386252     |
| KBP-COVID-19       | Protein subunit     | 1/2   | Observer-blind, dose-finding randomized | 180       | Not Provided           | NCT04473690     |
| Inovio INO-4800    | DNA                 | 1/2   | Dose-finding, Open-label (A), double-blind (B) randomized | 160       | Republic of Korea      | NCT04447781     |
| Arcturus ARCT-021  | RNA                 | 1/2   | Double-blind randomized               | 92        | Singapore              | NCT04480957     |
| AnGes AG0301-COVID19 | DNA             | 1/2   | Dose-finding, Open-label non-randomized | 30        | Japan                  | NCT04463472     |
| Themis V591        | Replicating viral vector | 1/2   | Dose-finding, double-blind randomized | 260       | USA, Austria, Belgium | NCT04498247     |
| Inovio INO-4800    | DNA                 | 1/2   | Dose-finding, open-label (A), double-blind (B) randomized | 160       | Republic of Korea      | NCT04447781     |
| Novavax NVX-CoV2373 | Protein subunit   | 1/2   | Dose-finding, observer-blind randomized | 1,419     | Australia, USA        | NCT04368988     |
| Imperial LNP-nCoVsaRNA | RNA          | 1     | Dose-finding partially randomized     | 320       | UK                     | ISRCTN17072692  |
| Medicago CoVLP     | Virus-like particle | 1     | Dose-finding, open-label randomized    | 180       | Canada                 | NCT04450004     |
| Curevac CVnCoV     | RNA                 | 1     | Dose-finding, single-blind randomized  | 284       | Belgium, Germany       | NCT04449276     |
| PLA-AMS ARCoV      | RNA                 | 1     | Dose-finding randomized               | 168       | China                  | ChiCTR2000034112 |
| Moderna mRNA-1273  | RNA                 | 1     | Dose-finding, open-label non-randomized | 120       | USA                    | NCT04283461     |
| Clover SCB-2019    | Protein subunit     | 1     | Dose-finding, double-blind randomized  | 150       | Australia              | NCT04405908     |
| BioNTech BNT162    | RNA                 | 1     | Double-blind randomized               | 144       | China                  | NCT04523571     |
| Inovio INO-4800    | DNA                 | 1     | Dose-finding, open-label non-randomized | 120       | USA                    | NCT04336410     |
| University of Queensland vaccine | Protein subunit | 1     | Dose-finding, double-blind randomized | 216       | Australia              | NCT04495933     |
| Symvivo bacTRL-Spike | DNA               | 1     | Dose-finding, observer-blind randomized | 12        | Australia              | NCT04334980     |
| Cansino Ad5-nCoV   | Non-replicating viral vector | 1     | Dose-finding, open-label non-randomized | 108       | China                  | NCT04313127     |
| SGMI aAPC          | Other               | 1     | Open-label non-randomized              | 100       | China                  | NCT04299724     |
| SGMI LV-SMENP-DC   | Other               | 1     | Open-label non-randomized              | 100       | China                  | NCT04276896     |
| Themis V591        | Replicating viral vector | 1     | Dose-finding, double-blind randomized  | 90        | Belgium, France        | NCT04497298     |
| Gamaleya Gam-COVID-Vac (Lyo) | Non-replicating | 1     | Open-label non-randomized              | 38        | Russia                 | NCT04437875     |
| AZLB protein subunit vaccine | Protein subunit | 1     | Double-blind randomized               | 50        | China                  | NCT04445194     |
| Medigen MVC-COV1901 | Protein subunit    | 1     | Dose finding, open-label non-randomized | 45        | Taiwan                 | NCT04487210     |
| Vaxine protein subunit vaccine | Protein subunit | 1     | Double-blind randomized               | 40        | Australia              | NCT04453852     |

aAPC, artificial antigen presenting cell; AZLB, Anhui Zhifei Longcom Biopharmaceutical; BIBP, Beijing Institute of Biological Products; CAMS, Chinese Academy of Medical Sciences; KBP, Kentucky BioProcessing; LV-SMENP-DC, vaccine comprising dendritic cells (DCs) modified with lentivirus (LV) vectors expressing ‘SMENP’ minigene; PLA-AMS, People’s Liberation Army Academy of Military Science; SGMI, Shenzhen Geno-Immune Medical Institute; WIBP, Wuhan Institute of Biological Products.
chemokine-dominant hypercytokinemia, often called a ‘cytokine storm,’ which robustly promotes expression of numerous IFN-stimulated genes that lead to multi-organ failure (Zhou et al., 2020). Therefore, SARS-CoV-2, directly and indirectly, triggers the impairment and hyper-stimulation of the immune system (Jamililoux et al., 2020; Yazdanpanah et al., 2020). But, cellular immunogenicity, humoral, and cell-mediated immune responses are crucial for vaccine-derived immunity and rapid cytotoxic response against viral infection (Morris et al., 2016; Ewer et al., 2017). Thus, should vaccines stimulate or suppress the immune response system of the host against COVID-19 infections?

**WILL A VACCINE PROVIDE SUSTAINABLE IMMUNE ENDURANCE?**

Another major concern about immunity against coronaviruses is the endurance of the immune response system. For effective immunization, vaccine-induced long-term regulation of the immune system, especially humoral and cell-mediated arms of the adaptive system, functions through producing the effector cells for the current infection and memory cells for future infections with the pathogenic agent (Clem, 2011). However, a number of studies showed that immune responses against COVID-19 do not last long-term. A study conducted on 285 SARS-CoV-2-infected persons reported that antiviral immunoglobulin-G (IGG) and IgM were increased during the first 3 weeks after symptom onset, and then began to decrease (Long et al., 2020a). A case report of 34 hospitalized patients (admitted from Feb 1 to Feb 29, 2020) with confirmed SARS-CoV-2 revealed that IgM levels reached their peak after three weeks and then continued to decline up to the end of 7 weeks of observation, whereas IgG values remained more or less the same (Xiao et al., 2020). In another case report, both IgM and the IgG declined after the peak period; this study was conducted on 60 convalescent patients where the value of those two antibodies reached their summit 6-7 weeks after onset, and a decline was observed in the following week (Du et al., 2020). A report from the National COVID Scientific Advisory Panel of the UK mentioned that IgG titers increased within three weeks of the onset of symptoms and started to drop by eight weeks in plasma samples collected from 40 confirmed COVID-infected persons (Adams et al., 2020). Another study noted that the most plasma samples obtained from eight volunteers infected with homologous virus (Callow et al., 1990). In cases of asymptomatic infection, a study conducted on 37 individuals reported that they had a weaker immune response, i.e., a greater reduction of IgG and neutralizing antibody levels (Long et al., 2020b). These phenomena are not new to the scientific world. Exactly 30 years ago, in 1990, a study reported a similar result. From an investigation of circulating lymphocyte populations in 15 volunteers infected with a CoV 229E strain, the researchers observed that the concentration of antibodies began to rise one week after inoculation and then reached their peak another week later. After that, titers of the antibody began to decline. They also claimed that despite the slightly high concentration after one year, this did not always prevent the volunteer from being reinfected with homologous virus (Callow et al., 1990). Thus, how long will a vaccine-mediated immune response be sustained and at what magnitude?

**HOW WILL SARS-COV-2 MUTATE?**

The genome of coronavirus is highly susceptible to mutations that result in genetic drift and evade immune recognition. Several studies have described this phenomenon. The genetic analysis of 86 complete or near-complete genomes of SARS-CoV-2 disclosed many mutations and deletions in coding and non-coding regions (Phan, 2020). High-resolution mapping of the SARS-CoV-2 transcriptome and epitranscriptome found at least 41 potential RNA modification sites with an AAGAA motif (Kim et al., 2020). A study of 95 complete genome sequences found 116 mutations including the three most common mutations, i.e., 8782C>T in ORF1ab, 28144T>C in ORF8, and 2905C>T in the N gene (Khailany et al., 2020). Mutations are also found in the S protein region, the crucial part for binding to human receptor ACE2. Another study reported that five of the six receptor binding domain residues of the S protein of SARS-CoV-2 differ from SARS-CoV (Andersen et al., 2020). However, this transformation does not stop there. A study identified 13 mutations in the S protein especially in spike D614G, which began to spread in Europe in early February 2020. This study also showed the evidence of recombination between the locally circulated strains indicating the multiple strain infections. (Korber et al., 2020). Twelve distinct variants were identified within the B-cell epitopes of the S protein, N protein, and M protein, and 21 distinct variants within T-cell epitopes. Of the 12 variants in the B-cell epitopes, 23403A>G Variant (p. D614 G) in an S-protein epitope has frequently been found in European countries such as the Netherlands, Switzerland and France, but rarely seen in China (Koyama et al., 2020). However, SARS-CoV-19 might not be evolving as rapidly as other RNA viruses, but we still need much more scientific evidence. Nevertheless, rapidly evolving viruses such as influenza need to be monitored to recommend new vaccine formulations twice each year (Gerdil, 2003). Similarly, no human immunodeficiency virus vaccines exist yet (Andrews and Rowland-Jones, 2017). In these circumstances, will the genetic stability of the SARS-CoV-19 remain such an extent that let the scientists develop a safe and effective vaccine?

**ARE WE PREPARED FOR VACCINE BACKFIRES?**

In this pressing time, perhaps some drug makers will rush through small-scale human tests that might not provide sufficient scrutiny of side effects or backfires. However, no vaccination is entirely free of any side effects or complications, and most are preventable illnesses (Kimmel, 2002). Recently, the phase 1/2 clinical trial of the ChAdOx1 nCoV-19 (NCT04324606) vaccine against COVID-19 also reported side effects such as fever, pain, muscle aches, chills, headache, and uneasiness. The research team claimed that these effects can be reduced by prophylactic paracetamol (Folegatti et al., 2020). Nevertheless, extreme caution must be taken to scrutinize backfire-effects i.e. the undesirable adverse effects (Table 2). One such dangerous backfire is vaccine-induced enhancement, which has been a major bottleneck in the development of certain corona-, flavi-, lenti-, and paramyxovirus vaccines. Here, antibody-dependent enhancement (ADE) per-
EFFECTIVE DEVELOPMENT STRATEGIES

The design and development of an efficacious vaccine is always a complex work, particularly in the case of SARS-CoV-2 that already have been mentioned in the previous sections.
indeed required to reach the finishing point successfully. The end, in every hurdle race, sensibility, skill, and speed are sufficient amount of data (Keshavarzi Arshadi candidates. Therefore, the computational models may help us to need huge amounts of data to be evaluated, analyzed, and validated, AI could do the work with minimal cost, time, and effort compared to the existing setup. Besides, in silico method has already been used in developing drug and vaccine candidates. Therefore, the computational models may help us to find the candidate vaccines and therapeutics if it is fed with a sufficient amount of data (Keshavarzi Arshadi et al., 2020). In the end, in every hurdle race, sensibility, skill, and speed are indeed required to reach the finishing point successfully.

CONCLUSIONS

The number of morbidities and mortalities related to COVID-19 is increasing day by day. Global and local economies are on the verge of depression, which is exacerbating humanitarian crises across the globe. Most of the countries have imposed lockdown and stay-at-home-strategy to break the chain of the community transmission; however, these preventive methods are not sustainable for a long time. As such, there is a dire need for a vaccine against COVID-19. An efficient vaccine is the best option for controlling and prevention COVID-19 pandemic. Addressing the raised questions in this paper will improve the safety and efficacy of any COVID-19 vaccine.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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