COVID-19 vaccines: Where do we stand?

Pinar Tulay^{1,2*}, Mahmut Cerkez Ergoren^{1,2}, Munis Dundar³

Abstract

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) was first reported in the city Wuhan, China in December 2019. The high rates of infection led to quick spread of the virus around the world and on March 11th, 2020, the World Health Organization (WHO) announced the pandemic of the Coronavirus disease 2019 (COVID-19) caused by the SARS-CoV-2. The pharmaceutical companies and institutions have been working towards developing a safe and effective vaccine in order to control the pandemic. The biology of SARS-CoV-2 is briefly discussed describing the transcription of the virus and the receptor recognition. The spike protein of SARS-CoV-2 is important in the attachment of the host cell and RNA-dependent RNA polymerase (RdRp) is involved in the replication of the virus making them good candidates for drug and vaccine targets. To date many different strategies have been employed in the development of vaccines and a number of them are in the phase III of clinical trials with promising results. In this mini-review, we assessed the literature thoroughly and described the latest developments in SARS-CoV-2 vaccines for humans. The main benefits and drawbacks of each platform is evaluated and the possible changes in the vaccine effectivity due to naturally occurring SARS-CoV-2 mutations have been described.

Introduction

The Coronavirus Disease-2019 (COVID-19) had arisen with the quick spread of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Upon this quick rise in the number of affected individuals worldwide, World Health Organization (WHO) announced the pandemic of COVID-19 on March 11th, 2020 (1). The manifestation of COVID-19 affected individuals changes from mild to critical conditions. Furthermore, a number of SARS-CoV-2 affected patients manifest the disease asymptomatic, whereas the most common symptoms are dry cough, fever and in some cases bilateral pneumonia (2). Furthermore, the older males with comorbidities are at an increased risk of being affected by SARS-CoV-2. More than 37 million COVID-19 cases with one million deaths had been reported by October, 2020 (3).

SARS-CoV-2 belongs to the coronavirus family (CoVs) that is a subfamily of *Coronavirinae* in *Coronaviridae* family. The CoVs are single-stranded and positive-sense RNA molecule. The genome of SARS-CoV-2 is surrounded by the nucleocapsid protein (N) forming the capsid and enveloped by the structural proteins of spike protein (S), membrane protein (M) and envelope protein (E), respectively (4). SARS-CoV-2 recognizes the host cell by the receptor binding domain through the receptor angiotensin-converting enzyme 2 (ACE2). The spike glycoprotein (S) facilitate the entry into the host cells making it an important target for antiviral treatments. The S protein consists of two subunits, S1 and S2, respectively. S1 subunit functions in the binding on the host cell's receptor; whereas S2 subunit is involved in the fusion of the virus' and host cell's membranes (5,6). S1/S2 boundary of SARS-CoV-2 consists of furin cleavage site consisting of four residues. These four subunits have the minimal polybasic fusing cleavage site of RXYR. This polybasic

¹Near East University, Faculty of Medicine, Department of Medical Genetics, Nicosia North, Cyprus

²Near East University, DESAM Institute, Nicosia North, Cyprus

³Erciyes University, Faculty of Medicine, Department of Medical Genetics, Kayseri, Turkey

*Corresponding author:

Assoc. Prof. Pinar Tulay, Ph.D.
Near East University, Faculty of Medicine,
Department of Medical Genetics
Near East University, DESAM Institute, Nicosia,
Cyprus
Near East Boulevard, Nicosia North, Cyprus
E-mail address: pinar.tulay@neu.edu.tr

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cleavage site is not found in SARS-CoV-related betacoronaviruses in humans. Thus, it is a possibility that the high rates of pathogenesis is due to the necessity of the furin proteases to be activated by the proteolysis of S (7). The replication/transcription complex, with viral non-structural proteins and RNA-dependent RNA polymerase (RdRp), mediate the SARS-CoV-2 replication. Thus, RdRp is another attractive antiviral target. The main protease (M^{pro}) is also involved in the replication and the transcription making RdRp another good candidate for developing antibodies, drugs and vaccines.

In order to control the pandemic of COVID-19, the need for the development of effective vaccines has become the priority worldwide. WHO enabled the collaboration among research community and institutes worldwide. To date, different forms of vaccines; including DNA- and RNA-based vaccines, adenoviral vector vaccine, inactivated vaccine and subunit protein vaccine; are in the process of being developed (8). Furthermore, a number of these vaccines are in the human clinical trial stage, including the non-replicating Ad5 vectored COVID-19 vaccine by CanSino Biologics Lnc, DNA vaccine by Inovio Pharmaceuticals, mRNA vaccine by BioNTech and ChAdOx1 nCoV-19 by Oxford University, respectively (9). In the next section, these different types of vaccines that are in different stages of clinical trials are reviewed.

Inactivated viral vaccine

Inactivated viral vaccines are composed of virus particles where the viral replication has been blocked. However, these viruses can still induce the immune response (10). The virus is inactivated by heat radiation or chemicals. Upon administration with appropriate adjuvants, inactivated viral vaccines activate the immune system. The animal studies of the inactivated viral vaccine, PiCoVacc (NCT04456595), was shown to stimulate the neutralizing antibodies which then promoted the antibodies against the spike protein of the virus (11,12). There was no adverse effects, such as fever or weight loss, in the animal studies. Furthermore, the phase I and II clinical trials showed that antibodies are activated 14 days post vaccination with no adverse outcome. The phase III clinical trial is aimed to be completed by October 2021 (13). The Sinovac Biotech has the permission to use this vaccine in high-risk groups in order to control the outbreak. Another inactivated viral vaccine was also developed by Sinopharm (ChiCTR2000034780) showing successful promotion of neutralizing antibodies with adverse effects of mild pain at site of injection and a number of subjects with fever. The phase III clinical trials for this inactivated viral vaccine is aimed to be completed by July 2021 (13).

Non-replicating viral vaccine

The viral vectors that are replication-defective is being used as non-replicating viral vaccines. Thus, even though these vaccines infect the human cells, it does not cause the disease. Adenoviruses are the common non-replicating vectors. These viruses have the same effect as the natural virus stimulating viral protein production in the host cell. Ad5 vectored vac-

cine (ChiCTR2000030906) aims to target the spike protein. The phase I clinical trial of this vectored vaccine included 108 healthy subjects, ages between 18 and 60 years. The subjects were divided in three groups of 36 who were administered different doses. In each group, minimum of one side effect was announced in the 7 days upon vaccination. The most common adverse outcome was the pain at the site of injection. Additionally, up to 46% of the patients presented fever and fatigue. Further results showed that there was no severe symptoms in the first 28 days post-vaccination. The reports concluded that the adverse symptoms were not severe. Furthermore, the responses of T-cells were shown to be as rapid as 14-days following vaccination. The phase III clinical trial of this vectored vaccine is the first vector-based vaccine at this stage of the development. Furthermore, Oxford University and AstraZeneca pharmaceuticals are in the phase III trial of a non-replicating viral vaccine development, named as ChAdOx1 nCoV-19 (NCT04324606) (13). This vaccine uses the simian adenovirus vector ChAdOx1 with SARS-CoV-2 spike protein and plasminogen activator leader sequence (14). In the phase I and II clinical trials, the vaccine showed induction of neutralizing antibodies with no severe adverse effects. The phase III clinical trial is aimed to be completed by October 2021. Another non-replicating viral vaccine is being developed by Johnson & Johnson's Janssen using the adenovirus serotype 26 (Ad26) adenoviral vector (NCT04436276). Initial results of this vaccine prove to be safe and eliciting the neutralizing antibodies (15). Phase I and II clinical trials are being evaluated aiming to be completed by 2023 (13). The Gamaleya Research Institute also developed a non-replicating viral vaccine using both Ad5 and Ad26 with spike protein. Even though, there is no published data on the phase I and II clinical trials, phase III trial is initiated with an estimated completion date of August 2020 (13).

DNA-based vaccine

DNA-based vaccines stimulate B and T cell responses *via* introduction of engineered DNA plasmids. Upon injection, the host cells start the plasmid-encoded gene expression stimulating immunization (16). INO-4800, DNA-based vaccine, has shown neutralizing antibody response in animal studies. Clinical trials of INO-4800 has been initiated and the administration is performed by a device, named CELECTRA®. This device enables the entry of the plasmids into the cell via an electrical pulse opening the cell pores (13). Another DNA-based vaccine, named GX-19 (NCT04445389), uses a similar delivery strategy of electroporation. Animal studies showed promising results of neutralizing antibody production. Clinical trials are in the progress aiming to have the initial results of adverse effects and T cell immune response by June 2022 (13).

mRNA-based vaccine

mRNA-based vaccines have recently being developed aiming to copy the natural infection. The mRNA-based vaccines are designed to deliver synthetic mRNA strand. The delivery of the mRNA is enabled by nanoparticle encapsulation (17). The main

difference between the mRNA-based vaccine and the conventional vaccines is the use of synthesized non-viral mRNA. The mRNA encoding the viral spike protein upon fusion is aiming to stimulate the antiviral response. Upon intramuscular injection, the mRNA-based vaccine is designed to specifically target the spike protein (18). The Moderna's mRNA-based vaccine, named as mRNA-1273 (NCT04283461), has been tested in clinical trials and completed both phase I and phase II trials. In the manufacturing of this vaccine, RNA with no replication ability is encapsulated in lipid nanoparticle that is encoding the spike protein. The aim is to induce the expression of the spike protein in prefusion conformation in the host cells resulting in the immune response. Phase I clinical trials included 45 healthy subjects, ages between 18 and 55 years, showing a strong neutralizing antibody response as well as cellular immune response. Furthermore, the results showed no severe safety issues with mild symptoms, including fatigue, headache, chills and pain at the site of injection (19). The phase II clinical trials aimed in assessing the dose-conformation and assess the safety, immunogenicity and reactogenicity. The phase III clinical trial (NCT04470427) was initiated in July, 2020 aiming to test 30,000 participants.

The collaboration of BioNtech and Pfizer and Fosun Pharma also resulted in two mRNA-based vaccines, BNT162b1 and BNT162b2. The BNT162b2 showed a less systemic reactogenicity and thus phase II and III clinical trials are continuing with BNT162b2 with an estimated completion date of November 2022 (13). Another phase III clinical trial of mRNA-based vaccine is developed by the Suzhou Abogen Biosciences, Academy of Military Sciences and Walvax Biotechnology Company showing strong neutralizing antibodies in animal studies (13).

Protein subunit vaccine (adjuvanted recombinant vaccine)

Protein subunit vaccines induces the immune response by introduction of adjuvants. These vaccines are expected to block the binding of the virus and membrane fusion (14). Chongqing Zhifei Biological China has initiated the trials with the protein subunit recombinant protein vaccine (NCT04466085) with an expected end date of December 2021 (13).

Does mutations in SARS-CoV-2 change the vaccine effectivity?

Higher mutation rates are detected in the RNA viruses compared to the DNA viruses, respectively. The changes of the surface protein's amino acid sequence cause alterations in the function of the virus and it also alters the interaction with the neutralizing antibodies. A number of spike protein mutations have been reported (20–22). Even as early as May 2020, 329 variants in spike protein that occurred naturally were stated. The D614G mutation is the first identified mutation in Wuhan followed by Europe in February 2020. This is shown to be the main mutation identified globally. It is suggested that spike protein undergoes conformational change due to this variant leading to higher infectivity (23). Spike S1 deletion

within the N-terminal domain (NTD) have been identified. Scarce number of studies investigated the effect of these variants on the function of neutralizing antibodies showing gain in resistance against some of the monoclonal neutralizing antibodies. These variants with gained resistance include L452R, N439K, V483A, A475V, F490L, Y508H, D614G+A435S and D614G+I472V. It has been shown that nearly all of these naturally occurring variants are within the RBD site. Furthermore, variants, either with one amino acid change, such as Q414E, Y145del, G446V, N439K, I472V, K458N, T478I, A475V, F490L, V483I, and A831V, and two changes in the amino acid, such as D614G+I472V, D614G+Q321L, D614G+A879S, D614G+A831V and D614G+M1237I, were shown to gain resistance against convalescent sera (24).

Discussion

The rapid spread of the COVID-19 has alerted the scientists to develop a reliable, efficient and safe vaccine aiming to get the pandemic under control. The pandemic has emerged endangering the human life and health and burdening the economy worldwide. The inadequate experience and molecular knowledge of SARS-CoV-2 has limited the rapid development of drugs or vaccines. The research institutes and pharmaceutical companies have joined together to develop the most effective and safe vaccine in order to overcome the pandemic. Development of vaccines has been proven to be difficult due to inconsiderate design of the vaccine and/or viral mutation.

A number of different strategies have been employed in the development of SARS-CoV-2 vaccine, in which each has benefits and drawbacks. The advantage of using viral vectors include the possibility of antigen-specific cellular and humoral immune response activation. Furthermore, these vectors do not require adjuvants during delivery with a chance of excessive production possibility. However, the immune system of the host may lower the effect of the virus (25). The other strategy in vaccine development is the use of adenoviral vectors. These vectors are being used in 20% of all gene therapies. However, they may have restrains in the immune system reactions, vector packaging capacity and viral longevity. The development of inactivated vaccines use the pre-existing technology and thus it has been used for many diseases, including SARS-CoV-1 vaccinations. However, these vaccines require booster doses with large amount of viral handling (26,27). DNA based vaccines can be produced easily. Furthermore, DNA is heat stable and the use of infectious viral particles is not necessary. However; DNA plasmids have been shown to remain in the host cells up to two years in animal studies (28). Furthermore, cytokines are being used to improve the immunogenicity of DNA and this could lead to adverse outcomes of immune suppression or autoimmunity. There is also a possibility that it may integrate into the human genome leading to anomalies. To date, although there have been many clinical trials of DNA vaccine for different viral infections, there is no licensed DNA vaccine in human use. With mRNA-based vaccines, viral handling is not necessary. Furthermore, since mRNA is translated in the cytosol, the risk

of host genome integration is low. However, booster doses may also be required for these vaccines and reactogenicity may be a concern. Protein subunit-based vaccines have the main advantage of being able to be used in immunocompromised patients. The use of infectious viral particles is not required. However, they may have low immunogenicity.

In conclusion, to date many have united to develop a safe and efficient vaccine in the anticipation of mitigating the pandemic. As discussed previously, different platforms to enhance the most effective way of immune response with none or mild adverse effects are in the process of being developed. There are multiple platforms at different stages of clinical trial with promising results. However, the long term safety concerns and variable effectivity due to naturally occurring mutations remain.

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