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TOWARDS SUFFICIENT IMMUNITY: INSIGHT INTO SARS-COV-2 VACCINES BASED ON VIRUS STRUCTURE AND PROPERTIES

Fatemah Salem Hassan Basingab[1,2], Kawther Sayed Ali Zaher[2*]

1. ***Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, Jeddah, KSA.***
2. ***Immunology Unit, King Fahad for Medical Research. King Abdulaziz University, Jeddah, KSA.***

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ABSTRACT

The world is currently still overwhelmed with coronavirus family that resembles MERS-CoV and SARS-CoV. A new virus variant, SARS-CoV-2, appeared in China in 2019 and still creating a global pandemic. Scientists have developed traditional and innovative vaccine strategies to achieve herd immunity status and contain the viral spread. Gene-based vaccines that rely on viral vectors, RNA, and DNA can provoke both humoral and cell-mediated immune responses, supporting their potential usage in developing COVID 19 vaccines. FDA has authorized the use of RNA-based vaccines against SARS-CoV. This review aims to compare the available gene-based COVID-19 vaccine considering their administration methods, antigenic determinants, and delivery routes. It also highlights clinical trials summary of each vaccine technology along with its advantages and disadvantages. The study discusses the evolution of SARS-CoV-2 variant and compares this virus with its ancestor. Moreover, the study reviews critical elements of gene-based vaccine advances against several infectious diseases. In conclusion, this study is a guide for the progress of successful vaccines against future SARS-CoV-2 derivatives.

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Introduction

A new coronavirus variant, SARS-CoV-2, was initially discovered in China in 2019 also the biological cause of COVID-19 disease [1, 2]. World Health Organization (WHO) has published instructions to contain the extent of this virus through isolation strategies. The fast extension of the virus to hundreds of counties resulted in labeling it as a pandemic [3]. The distribution and epidemiology of SARS-CoV-2 have varied intensively. The epidemic primarily affected China in Asia, then soon spread to Italy and several other nations in America and Europe [4]. Likewise, the mortality rate (MR) of 3.4% was estimated by the WHO. However, the MR differed regionally based on the regional capacity of the health system and the individuals' health status and vulnerability. In addition, earlier diagnosis can result in better outcomes; therefore, efficient and testing COVID-19 strategies also had an impact [5]. SARS-CoV-2 can also be spread through drops within two meters during coughing, sneezing, and even speaking. SARS-CoV-2 contaminated surfaces or asymptomatic individuals may also transfer the SARS-CoV-2 during exposure. SARS-CoV-2 has a 2-14 day incubation period, with severe symptoms developing within 7-10 days. Fever, dry cough, tiredness, and/or diarrhea were common COVID-19 symptoms. Approximately 20% of those infected, especially the aged and people who are suffering from underlying health conditions, required hospitalization. The signs can lead to severe chest pain and pneumonia [6].

Virus Structure

Coronavirus was first diagnosed in a child suffering from common cold-like symptoms in 1965. The given name "corona" came from the viral crown-like appearance under electron microscopy representing the viral spikes radiating at the surface. SARS-CoV-2 comprises of enveloping S and M glycoproteins. The spike "S" glycoprotein is responsible for viral attachment to host cell receptors and facilitates viral fusion. The "M" is a membrane glycoprotein that is necessary for the viral envelope formation and assembly [6]. The virus is a single strand RNA with a positive sense range from 26-32 Kbp (with an average of

Corresponding Author: Kawther Sayed Ali Zaher; Immunology Unit, King Fahad for Medical Research. King Abdulaziz University, Jeddah, KSA. E-mail: kzaher@kau.edu.sa.

29.8 Kbp) with a 5′-cap structure and 3′-polyA tail. At the 5′ terminal, 7-14 open reading frames (ORF) encode 29 proteins. Transcription sequences for regulation containing transcription termination and acquisition of leader RNA are between them. ORF1a and ORF1b genes are sub-genomic RNAs that form a duplication transcription complex during transcription of genomic RNA; their crucial function is to produce a nested set of non-structural proteins [6, 7]. Spike (S) protein, envelope (E) protein, membrane (M) protein, and nucleocapsid (N) protein are the four structural proteins encoded by the 3′ genome terminus [8]. The S protein (150 kDa) helps the virus to connect to the host cell membrane, which contains receptors for viral entrance via the fusion process, enhancing the virus attachment. The S protein is the most dominant Immuno-ectodomain protein, which activates immune response comprises of two efficient subunits; S1 binds to the cell host receptor, and S2 fuses coronavirus with cellular membranes. The trimeric S1 localizes on the trimeric S2 stalk's apex [9]. The arrangement of unique parts of S1 differentiates dissimilar coronavirus [10]. The N terminal domain (NTD) and the C terminal domain combine to generate S1 (CTD). The receptor-binding motif (RBM) found in CTD binds to its specific receptor, composed of peptide area of angiotensin-converting enzyme2 (ACE2) [11]. RBD is the variable part of the CoVs genome [12] **(Figure 1)**. M protein (approximately 25–30 kDa) is a structural protein that determines the viral envelope's shape. Together M and S (NTD) proteins interact to diffuse into the Endoplasmic Reticulum (ER) and Golgi apparatus (GI) to make a complex called (ERCIC) during replication to form new virions [13, 14]. The E protein is a minor structural protein (approximately 8–12 kDa) where its primary function is during assembly and budding and completing virions [15]. N protein is critical because it forms a complex with the viral genome to facilitate the M protein role through virus assembly and facilitates viral transcription [16]. It comprises three highly conservative domains. The first domain is the NTD domain which binds with 3′ terminus via electrostatic interaction, and this domain is extremely swerved in length and sequence. The second domain is LKR, RNA- binding domain or linker, responsible for upsetting cell signaling and antagonizing interferon [17].

Origin of SARS-CoV-2

Coronaviruses (CoVs) generally cause intestinal and respiratory affection symptoms in both humans and animals [18]. The subfamilies of this virus were defined as alpha, beta, gamma, and delta. It is known that beta coronavirus affects bats. Bats are the main reservoir of alpha and beta coronavirus and transmit various SARS-like-CoVs. Such bats are spread all over the globe, especially in China. Researchers have been devoted to studying the genetic variation of such viruses and their development [19].

In 2002-2003, typical pneumonia cases were reported in Hong Kong where the new CoV virus was isolated and the so-called SARS-CoV (severe respiratory syndrome coronavirus). After then it extended to 8000 and more people in 26 nations with 10% causality. Spread to people was through an intermediate host raccoon dog [20]. In 2003, the disease vanished, and no case was reported [21].

Another famous CoV originating from an animal source is Middle East respiratory syndrome coronavirus (MERS-CoV), that causes more deaths. Still, it is known to be rarely spread between humans. In 2012, It was initially recognized in Saudi Arabia. It is believed that the disease was spread from camels infected by the bat to humans, where camels acted as intermediate hosts [22].

New variant coronavirus-2019 (SARS-CoV-2) was primarily described in Wuhan City in China, which resulted in the ongoing pandemic of Covid-19 [23]. The epidemic was initiated at the end of 2019, and currently, 22 million and more people have been identified with the infection, and 200 and more countries have been impacted by the virus. Also, the virus has led to approximately 785,000 casualties. Later on, the disease subsided in China, but it spread to the rest of the Globe viciously. Now, new strains of SARS-CoV-2 have spread into different countries [24]. The viral source is proposed to be bats as it is the natural reservoir. However, a recent study suggested pangolins are responsible for it as a result of the high similarity of all genome sequences between both pangolins- CoV and SARS-CoV-2 (92.02%) [25]. First, it was supposed that the transmission of the virus was through food, but soon it was discovered that it was an airborne infection. The disease may cause respiratory problems among non-respiratory symptoms [26]. Even though there is a great resemblance between SARS-CoV and novel-CoV-2, there are dissimilarities in their transmission effectiveness related to nucleotide alteration in the S protein spike and its receptor-binding domain [27, 28]. SARS-CoV-2 is comparable to beta-coronavirus, which is well-known to be a source of severe fatal diseases. It is critical to know that coronavirus contains up to 10 ORF while SARS-CoV-2 contains only one intact ORF [29].

Comparison of the Coronaviruses

In humans, coronavirus infection is usually classified as respiratory diseases such as high fever, severe inflammation, cough. Still, in cases of SARS, MERS, and SARS-CoV-2; acute respiratory distress syndrome (ARDS) develops and leads to dysfunction of the internal organs and can result in death in some cases. The symptoms, progression, and prognosis are far more serious in SARS-CoV-2 cases [19, 30]. The disease transmission in SARS and MERS cases is via direct contact, fomites, or droplets infection, but in SARS-CoV-2, the organism remains active for a long time (days on the surface and hours in the air) [31]. Many researchers have proposed that the Cov-2 virus can cause infection for many hours in vaporizers and days on surfaces; thus, contamination of vaporizer and fomite could perform effective roles in transmitting SARS-CoV-2. Disturbed immune responses, resulting in impaired pulmonary gas exchange, can contribute to the disease's immunopathology. Our knowledge of pulmonary inflammation related to this type of infection could be enlightened by appreciating the relationship between coronaviruses and the host's immune system responses mainly the innate type [32].

Even though the novel SARS-CoV-2 genomic sequence and SARS-like coronaviruses have a lot in common, the SARS-CoV-

2 genome sequence has a distinctive cleavage site (furin-like) in the spike protein [27]. It is proposed that this cleavage site could affect the virus pathogenicity as well as the life cycle of the disease and may even serve as a treatment aim for inhibitors of furin. Compared to its predecessors, the extremely infectious existence of SARS-CoV-2 may be due to a mutation of stabilizing type that happened in the nsp2 protein-like endosome-related-protein domain. Likewise, the nsp3 proteins de-stabilizing mutation in SARS-CoV-2 near the phosphatase domain might suggest a probable mechanism that distinguishes it from other CoVs [33]. SARS-CoV-2 deaths are larger than the combined deaths of both SARS and MERS. The recent discovery of deletion of 832-nucleotide (nt) in ORF8, seems to minimize the virus's replication capability and contributes to diminished SARS-CoV-2 phenotypes, and is potentially related to viral pathogenesis. The probability of breaching the species boundary for the third time happened in the case of COVID-19 [34].

In comparison, the potential intermediate or transitional hosts are palm civet and the dromedary camel for SARS-CoV and MERS-CoV infection respectively [35]. Bats are considered as ancestral hosts for SARS and MERS. The human CoVs reservoir hosts e.g. HCoV-229E and -NL63 are also known to be of bats. Primary spread in cases of COVID-19 is basically by one of the following ways: it can be a direct or indirect spread from bats through transitional hosts, which is comparable to MERS and SARS. In the SARS outbreak, the occurrence prototype indicates that SARS-CoV emerged from bats and then transferred to the transitional host (civets) and introduced alterations to enhance civet ACE2 binding within the receptor-binding domain. Throughout their following introduction to people in markets, this civet-adapted virus facilitated further modifications that ended in the epidemic strain [36, 37]. Individuals lacking RBD mutations can potentially be infected straight from the reservoir host. The current bat coronavirus in circulation contains unique "assembled" spike S proteins that enhance human infection in the absence of mutations or variants. Several bat species around the world carry a huge number of coronaviruses [38]. Overall, a large number of CoVsare borne all over the globe by various species of bats. In addition to the possibility of mutation as a part of adaptation and recombination, high plasticity in receptor use can also lead to the regular inter-species spread of CoV from bats to individuals and animals. The bat CoVs' pathogenesis is mostly unclear since the majority of such viruses have not been examined [39].

The latest evidence existing on infection of MERS indicates that camels are the primary host reservoir, along with humans [40]. There may be no visible signs of infection in the infected dromedary camels, making it difficult to distinguish animals which in turn excrete MERS-CoV that can communicate the disease to individuals. Nevertheless, they will spill MERS-CoV through urine, milk, discharge of nose and eye, and feces and may be present in the fresh organs [41]. Llamas and pigs were reported to be prone to infection by MERS-CoV, suggesting the probability of MERS-CoV transmission in animal types, not only dromedary camels [42]. Viruses recognized as SARS-CoV-like were segregated from Paguma larvata (Himalayan palm civets) and Nyctereutes procyonoides (raccoon dogs) detected in a market of live animals (Guangdong, China), after the outbreak of SARS in China. There was a sequence of 29-nucleotide that was not detected in the majority of humans' isolates was retained by isolates of animals acquired from the market [20]. In recognizing the likelihood of inter-species spread in SARS-CoV, such results were significant. A host/pathogen coevolution is suggested by the greater variety and incidence of bat CoVsin this area relative to those in prior research. Similarly, in Chinese Rhinolophus sinicus (horseshoe bat) populations, SARS-like CoVs have been detected spreading [38].

The latest outbreak of COVID-19 could be comparable to China's SARS-CoV that occurred in 2002-2003 and Saudi Arabia's Respiratory Syndrome CoV of Middle East (2012) for their zoonotic spread and several resemblance in clinical manifestations [43]. Nevertheless, phylogenetic analysis of the beta-coronavirus lineage RBD (receptor-binding domain) reveals that nCoV-19 mostly fits into bat-originating SARS-like CoVsnamely bat-SL-CoVZC45 and -SL-CoVZXC21showing 88-89% resemblance **(Figure 1)**. Though it has 50% and 79%resemblance to SARS-CoV and MERS-CoV respectively [44]. It should be noted that while there are important genomic variations between these CoVs and the 2019-nCoV subclass when the primers & epitopes of antigens are not chosen carefully, cross-reactions in PCR occur or when SARS or other beta-coronavirus antibody measurements happen [45, 46]. Angiotensin-converting enzyme 2(ACE2) detected in the host cell surface and is usually attached to RBD found at the spike protein's C-terminal domain. This enzyme is predominantly disseminated in the pulmonary and gastrointestinal epithelium. Extreme infection can similarly happen in tissues expressing ACE2in high levels, including the lungs, intestines, kidneys, and blood vessels. Utilizing the Swiss-Model Software 33, a center and an external subdomain represent the structure of 2019-n CoV- RBD with three dimensions (Protein Data Bank ID: 2DD8), similar to another beta coronavirus RBD. Interestingly, the resemblance between the 2019-nCoV external sub-domain of RBD and SARS-CoV reveals that the 2019-nCoV likewise binds to ACE2 to be able to contact the host cell, as shown in **Figures 1b-1d** [46]. Also, some RBD remainders enabling the attachment of the 2019-nCoV to the ACE2 receptor have been disclosed in modeling studies for example; Gly485, Gln493Asn439, Asn501, and Phe486, which vary from SARS-CoV-RBD ones [46, 47].

Research confirms that 2019-nCoV has a more novel shared progenitor with BetaCoV/RaTG13/2013 depending on re-combination study and trees of phylogenetic since both viruses are in the same cluster. Furthermore, such cluster could be the product of convergent development or complicated re-combination procedures connecting minimally two types of viruses with distinct evolutionary pasts.

The two outside fragments of such clustered viral genome are identical to the bat CoVs ZC45 and ZXC21, containing nucleotides (nt) 1 to 13 521, and 23 687 to 30 079. ORF1a is included in the first and the second fragments containing the C-terminus of S-protein, E, ORF3, M, ORF7a, ORF6, N, ORF8, and ORF10. This result is similarly confirmed by the reconstruction of phylogenetic maximum likelihood (ML) trees, showing that Sarbecovirus is clustered in fragments from nt

1 to 13 521 and from nt 23 687 to 30 079Regardless, the middle fragment does not cluster with Sarbecovirus from 13 522 to 23 686 of the 2019-nCoV genome and RaTG13, according to the ML tree. It makes a novel branch in the phylogenetic tree, between Sarbeco-virus and an Un-classified coronavirus. Furthermore, additional primary research has indicated the RBM (receptor-binding motif) of these two genomes have just a small amount of sequence similarity [48-50].

This variation, as suggested by previous primary investigations, refers to possible other origins for the 2019-nCoV RBM encoding sequence [51].

This variation suggests a possible alternate origin for the encrypting sequence of 2019-nCoV RBM, as shown by other researchers. Interestingly, 2019-nCoV has a resemblance of 85.5 percent to 92.4 percent. Other authors found multiple putative sequences of pangolin Coronavirus [52]. More new studies showing the presence of many genetically related pangolin CoV lineages with 2019-nCoV promote the theory that pangolins have acted as a possible transitional host [53]. The data currently available do not completely clear whether the virus was spread instantly from bats to individuals or via indirect way through a transitional host, nor do they at present exclude convergent development as an alternate recombination theory to clarify the conflicting phylogenetic trees.

Consequently, the exact origin and source of the 2019-nCoV, which can be done only through better collecting and observing bat and other samples of a wild animal, requires further sequence data to validate. The topology of a phylogenetic tree having all the 2019-nCoV gene sequences of spike protein currently available shows high levels of similarities between human isolates, suggesting that only a small genetic variation exists for rapidly emerging RNA viruses, which is somewhat surprising [54].

However, these correlations may be the consequence of a comparatively new shared ancestor, implying that a recent occurrence was the emergence of the virus. In addition, studies showed that the spread of the inter-species virus was highly localized or small, likely a single case [10, 18, 55]. Recent exposure to humans is suggested by the great similarity in the sequence between the isolated viruses from diseased humans. In all, the position of Wuhan as the epicenter of the epidemic is further established by these results, and there are no other origins of this 2019-nCoV that could be detected [43, 52].

Vaccination

Vaccination is a means of avoiding numerous infectious diseases such as e.g., polio, measles, etc. These policy technologies vary from live pathogenic inactivation and attenuation to the supply of peptide antigens of synthetic type and recombinant antigenic protein, as well as viral particulate matter (VLPs), viral vector non-replications and replications, conjugates of polysaccharide-protein, and vaccines based on DNA or RNA nucleic acid. New vaccine types include antigenic and nucleic acid vaccines, which can be of the utmost importance to COVID-19 in addition to conventional all-pathogen-based vaccines.

Specifically, genetically modified RNA or DNA is used by the nucleic acid-based and dominant protective epitopes method for antigen coding, e.g. the S-protein on SARS-CoV-2, to evoke the reaction of the immune system against the virus. The continuing outbreak may be overcome by proposal plans depending on the characteristics of viral exposed protein's epitopes that can be identified by T lymphocytes and antibodies generated by B cells using the immunoinformatic strategy to identify protein epitopes for COVID-19 infection vaccine development. As a result, the whole 66 sequences of non-self in the genome of the virus were acquired that represented the best goals for vaccine advance because of their immunogenicity prospective [56].

As of 25 August 2020, 142 applicant vaccines established on different platforms in pre-clinical points of improvement have been published in the WHO landscape document: only 31 are allowed to continue under clinical evaluation [57]. A cooperative initiative to improve an adjuvant vaccine for COVID-19 has recently been publicized by two pharmaceutical companies, Sanofi and GSK. Sanofi contributes its COVID-19 antigen S-protein, while GSK contributes its adjuvant technology, which has been shown to diminish the quantity of protein necessary in each dose of the vaccine. In the later part of 2020, both already began Phase I and clinical trials. AstraZeneca, Oxford University, and its spinout company Vaccitech are collaborating as a model of academic-industry teamwork to simplify the prompt manufacture and supply of their vaccine candidate, ChAdOx1, depending on a non-replicating chimpanzee adenovirus vector. The construct of ChAdOx1 is designed to activate the development of the viral S protein that then stimulates the immunity to identify infection with SARS-CoV-2. AstraZeneca also has overseen the first Phase II/III clinical trial in the UK of ChAdOx1 nCoV-19, now called AZD1222, and performed in late 2020. China CanSino Biologics has developed an alternative candidate of vector-based vaccine, Ad5-nCOV, and is still undergoing clinical trials [58].

Inactivated Vaccines

Inactivated vaccines include a candidate for inactivated-COVID-19 vaccine produced by the Univ. of Osaka in Japan, which is comparable to their previous WNV (West-Nile-virus) inactivated vaccine [59]. Researchers at Colorado State University are evolving a COVID-19 inactivated virus vaccine (SolaVAX) depending on a current pathogen inactivation technique framework for products of blood, such as the use of ultraviolet rays and riboflavin by targeting nucleic acid injury while maintaining the integrity of the protein and the antigens of the virus [60, 61].

Sinovac Biotech (Beijing, China) and Dynavax (Emeryville, CA, USA) collaborated to create an amalgamation of Sinovac's COVID-19 vaccine candidate for chemical inactivation [62] and Dynavax's advanced adjuvant CpG 10188. Sinovac additionally demonstrated their full SARS-CoV-2 virus particles in phase I/II clinical trials using chemical inactivation (PiCoVacc) produced in VERO monkey cells and the adjuvant alum [58]. PiCoVacc prompted SARS-CoV-2-specific counterbalancing antibodies in rats, mice, and NHPs, providing NHPs with the best resistance against SARS-CoV2 [62].

The production of traditional vaccines with inactivation techniques includes the growth of highly infectious virus titers, that in the SARS-CoV-2 case, must occur at the level 3 biosafety facility, which is also chief safety distress. Furthermore, when the inactivation of the virus is incomplete, it presents a probable danger to vaccine manufacturing staff and can also lead to disease outbreaks in vaccinated people and bring an adverse response to the immune system.

Vaccines of Live Attenuated Type

An approach known as viral gene de-optimization has been used by Serum Institute (Pune, India) and Codagenix (Farmingdale, NY, USA) to produce a live-attenuated SARS-CoV-2 vaccine using balanced gene design with the use of computer and chemical synthesis [63]. Though live attenuated vaccines targeting viral infections that infect the respiratory system have been permitted for humans. The virus can shade in people infected with SARS-CoV-2. This raise concerns that a SARS-CoV-2 (live attenuated) vaccine strain could be shaded and spread to unvaccinated people. An additional disadvantage is the possibility of recombination between a live attenuated vaccinal virus and a wild-type -CoV [64, 65].

Subunit Vaccine

Subunit vaccines are made up of synthetic peptides or recombinant proteins from target pathogens. As a result, sub-unit vaccinations are often well-thought-out to provide a high level of safety. Subunit vaccines, moreover, can precisely aim for well-recognized neutralization of epitopes and the improvement of immunogenicity and/or efficacy in combination with adjuvants. Since the S-protein of SARS-CoV-2 has the main function in the attachment of receptors and it is suggested that vaccines with membrane fusion aiming at the S protein can induce neutralizing antibodies by blocking the viral attachment and fusion [66]. The S1 subunit, S2 subunits, NTD, and RBD, and the whole-length S protein and its parts, maybe significant antigen goals for the progress of subunit vaccines. GlaxoSmithKline (London, UK) and Sanofi Pasteur (Lyon, France) are working on a COVID-19 subunit vaccine applicant, with Sanofi subsidizing the antigen of S-protein using a baculovirus expression as a recombinant DNA technology. GSK is funding Adjuvant System 03 (AS03) as a novel adjuvant technology, which includes polysorbate 80, squalene, and dl-a-tocopherol. GSK, Clover Biopharmaceuticals, the University of Queensland (Brisbane, Australia), and Xiamen Innovax Biotech (Xiamen, China collaborated to test their adjuvants. A patent of Trimer-Tag technology, Clover Biopharmaceuticals promoted a SARS-CoV-2 S-Trimer subunit vaccine candidate (SCB-2019) that has a great similarity to intrinsic trimeric viral spike. Clinical trials with AS03 [67] or alum adjuvants and CpG 1018, SCB-2019, were successful in phase I [65]. Another vaccine depending on a chain of truncated S protein subunits is COVID-19 XWG-03 and is screened in combination with AS04. It is similar to Escherichia coli-produced subunit vaccines which were previously developed by Xiamen Innovax Biotech against HPV [68] in humans. Researchers at Texas Children's Hospital and Baylor College of Medicine (Houston, TX, USA) have examined SARS vaccine antigen based on the SARS-CoV S protein RBD to the candidate a SARS-CoV-2 vaccine that is equivalent. In addition, VIDO-InterVac at the University of Saskatchewan is testing a peptide-based COVID-19 vaccine candidate using a CpG oligodeoxynucleotides TLR agonist (a peptide of host defense) or polyinosinic-polycytidylic acid which is a mix of three adjuvant platforms (TriAdj) and polyphosphazene. With provision from the Alliance for Epidemic Preparedness Advances, Novavax (Gaithersburg, MD, USA) is conducting clinical trials for its patented candidate (subunit vaccine) of S protein antigens which was developed using recombinant technology platform using Sf9/baculovirus, as was previously done for an RSV vaccine candidate (CEPI, Oslo, Norway). The protein antigens were adjuvanted with a saponin-coupled with Matrix-MTM [66].

Non-replicating Viral Vector Subunit Vaccines

To transmit antigens of vaccine to the cells or tissues, viral vectors are used. A wide range of viral vectors that replicate and non-replicate are available. Adeno-related virus, alphavirus, and herpesvirus are vectors mainly designed as non or defective-replicating vectors, unlike replicating vectors as vesicular stomatitis virus, measles virus, and poliovirus, Janssen (Johnson & Johnson, Leiden, The Netherlands) uses AdVac R (based on adenovirus type-26) alone or mixed with MVA-BN R (Modified Vaccinia Ankara) virus technology) as a prime-boost immunization strategy against COVID-19 (Hellerup, Denmark). Artificial antigen-presenting cells that are pathogen-specific and LV-SMENP-DC are two lentiviral vector-based vaccination models under clinical processing at Medical Institute in China. For the lentiviral vector system (NHP/TYF) a vaccine called LV-SMENP-DC vaccine is utilized to express minigenes of SARS-CoV-2 into viral proteins. 190 Another vaccine is being established by Oxford University (Oxford, UK) and ChAdOx1 nCoV-19, an attenuated chimpanzee adenovirus made by Advent Srl (Pomezia, Italy) that may generate the S protein SARS-CoV-2 and is successfully anticipated to induce effective antibodies against SARS-CoV-2 proteins [66, 69].

Replicating Viral Vector Subunit Vaccines

The influenza, measles, horse pox virus and vesicular stomatitis viruses are used to generate platforms of viral vector to create new COVID-19 vaccine models. Southern Research (Birmingham, Alabama, USA) is collaborating with Tonix Pharmaceuticals (New York, NY, USA) to create a live modified horsepox virus (TNX-1800) designed for the production of S-protein SARS-CoV-2. While several candidates for COVID-19 vaccines prepared using viral vectors are in the preclinical and clinical stages of development, using viral vectors to deliver genetic vectors to cells has several drawbacks, including the viral vector itself can produce a non-desirable immunity in the body. Furthermore, if a vaccine fails to generate an immunological response during trials, the same vector cannot be reinjected in the patient. In addition, the vaccine's effectiveness may be hampered by previously built immunity to the viral vector. Finally, former immunity can be challenged

by utilizing increasing the vaccination dosage, modifying the administration route, or even by a non-viral DNA vaccine. Other disadvantages connected with viral vectors, such as genetic toxicity and decreased transgenic expression, can be mitigated by applying hybrid viral vectors [66].

DNA Vaccines

DNA vaccines consist of double-stranded plasmids that are designed to trigger a particular immune reaction in the host. In April, a candidate vaccine named as INO-4800, developed in partnership with Beijing Advanccine Biotechnology by Inovio Pharmaceuticals, established a small medical investigation in the U.S.A. [57].

Messenger RNA Vaccines

The technology of mRNA-based vaccines as a novel means in the production of prophylactic vaccination towards infections gained more interest in the last two decades [70]. Due to its effective, long-term, and healthy immune reactions perceived in models of animals and promising evidence from initial human clinical research, mRNA vaccination is an enticing alternative to traditional vaccine approaches [71]. Several mRNA-based vaccines, for instance, mRNA-1273 produced by Moderna and BNT162 (b1) produced by Pfizer Inc. and BioNTech SE, are currently being developed [57].

mRNA-1273 Vaccine

mRNA-1273 is a new LNP (lipid nanoparticle) condensed in mRNA vaccine encrypting for the spike (S) protein of prefusion stabilized type [72]. The vaccine phase 1 clinical investigation began in the 1st quarter of 2020 with 45 normal adults in a good health (18to 55 years of age) in three cohorts of doses (25, 100, and 250 μg) for six weeks in two doses approximately 4 weeks apart by upper arm intramuscular injection. Besides the initial volunteers, three groups of elderly volunteers (56 to 70-year-old) and three groups of stable elderly volunteers aged 71 and older were registered. Volunteers were monitored for a year after the 2nd vaccination to evaluate well-being data, shared symptoms of vaccination. A Phase II study in two cohorts of 600 healthy participants ranging from18 to 55 years old adults and 55 years adults and older adults received a 50 μg or 250 μg placebo dose as of 29 May 2020. In a Phase, I trial of open-label, and dose-range type, an NIAID-led mRNA-1273 study revealed a positive reaction from an initial report120, and Phase II/III 'COVE' studies are expected and performed early in the summer. The murine models in vivo experiments indicated that the vaccine causes an immune reaction and could induce S-binding antibodies from IgG2a and IgG1 subclasses. On re- evocation using peptide pools, mRNA-1273 immunized mouse splenocytes displayed greater gamma interferon (IFN-γ) secretion than other interleukins such as IL-4, -5, or -3 (S1 and S2). A dose of mRNA-127 of 1 μg induces a strong response of CD8+T cells to the S1 peptide pool with a balanced isotype response of Th1/Th2 antibodies in mice. A vaccine of 100 μg dose was then determined for a phase 3 human trial experiment corresponding to the dose of 1 μg introduced in mice [73].

BNT162 Vaccine

It is one of the four candidates of Pfizer development programmed licensed vaccine [74]. Two vaccines enclose mRNA encrypting for S-protein of SARS-CoV-2, whereas the other two, enclose only the spike protein RBD [75]. Moreover, three separate mRNA formats are made up of the four vaccine candidates. Two vaccines are grounded on mRNA adjusted by nucleosides (modRNA), which integrates adjusted nucleosides into the mRNA [76]. Acquired immune stimulation and the development of antibodies towards the mRNA are suppressed by mRNA itself. For lengthier periods, the decreased immune response towards the treating mRNA assistances production of the antigen. The third candidate vaccine is established on the format of optimized original mRNA (uRNA). In mRNA, uRNA utilizes uridine, giving it a more immunogenic nature. Lastly, the most recent candidate for the vaccine uses mRNA that self-amplifies (saRNA). It depends on the theory of viral replication. Along with encrypting a protein of interest, saRNA also encodes replication. This helps the mRNA inside the cell to self-amplify. The dsRNA intermediate produced during RNA replication makes saRNA induces a very strong immune response. BNT162 (b1) is both Phases I/II clinical study on 200 healthy adult volunteers is potentially efficient [77, 78].

Vaccine Application, Efficacy, and Safety

The Centers for Disease Control and Prevention (CDC) has issued guidelines of how to use mRNA COVID-19 vaccinations and anaphylaxis management are available [79, 80].

Precisely, sites dedicated for vaccination should secure the necessary supplies for managing anaphylaxis, particularly sufficient amounts of prefilled syringes of epinephrine or auto-injectors; also screen potential vaccine recipients for contraindications and precautions as well as follow recommendation post-vaccination observation periods, which should be 15 or 30 minutes depending on the vaccine. Patients who experience anaphylaxis should be admitted to a facility that can provide them with proper medical attention. All suspected patients should be reminded to ask for instant medical assistance if any signs of an unfavorable or undesirable reaction are observed after leaving the vaccination location. They should stay informed about the importance of reporting unfavorable events by using the Vaccine Adverse Event Reporting System (VAERS), which was declared by the Department of Health and Human Services/Centers for Disease Control and Prevention.

Possibilities that mRNA vaccines can cause type one interferon reactions, which might result in inflammation and/or autoimmune disorders [81], is one of the most serious hazards. The likelihood that targeting DNA into the DNA in chromosomes of the receptor may have mutagenesis consequences on the functional gene situated in the insertion loci is one of the safety issues with DNA-based vaccines [82]. At this time, mRNA and DNA-based vaccines have not been approved

against any disease for commercialization. In terms of using a delivery mechanism, such as a plasmid, to introduce selected DNA inside cells, where it is changed into proteins that stimulate an immunological response in the acceptors, resulting in besieged antibody responses and T-cell, DNA vaccines are analogous to genetic treatment. DNA is used for a variety of gene therapies, most of which are tied to inherited disorders or predispositions. Because of the severe dangers, the majority of gene therapists have said that gene therapy is appropriate solely for incurably ill patients. Vaccine delivery is very different from gene therapy interventions because the vaccine is specifically suitable for healthy individuals, and the analysis of the hazards and benefits they gain would be extremely different. Terminally ill people similarly to healthy people are at risk of introducing foreign DNA into their bodies. However, critically ill people may benefit from the opportunity to cure their disease. The healthy people may not value since they have never infected with the pathogen. Access to evidence after the trial has ended; the participants should be availed of the developed vaccine after the clinical vaccination trials are completed. This is one of the direct benefits of their participation in the study. Even though this vital requirement is contained in the international code of ethics, however not all researchers are familiar with it. COVID-19 vaccine development is currently taking place across many countries and continents, with participants coming from various countries and areas [83]

On December 11, 2020, the Food and Drug Administration granted an Emergency Use Authorization (EUA) for the Pfizer-BioNTech COVID-19 vaccine for COVID-19 in two doses, 21 days apart. The Advisory Committee on Immunization Practices (ACIP) has issued a preliminary recommendation for the Pfizer-BioNTech COVID-19 vaccine. The first recommended doses were for healthcare professionals and inhabitants of long-term care institutions on December 12, 2020. As of December 23, 2020, 1,893,360 introduction doses of Pfizer-BioNTech COVID-19 vaccine have been taken in the U.S.A., with 4,393 (0.2 percent) adverse effects described to the Vaccine Adverse Event Reporting System following administration of the vaccine (VAERS). 175 case reports, on the other hand, were acknowledged as severe allergic responses, including anaphylaxis, and were forwarded for further examination. Particularly that,70% of these cases occurred during the first 15 minutes of vaccination. The most successful approach is to manage anaphylaxis injection via an epinephrine injection in the muscles after implementing recommended post-vaccination observation periods [84].

The researchers looked at 12 174 ChAdOx1 nCoV-19 recipients and 11 879 control group members for significant adverse events. There were no notable adverse events or deaths associated with the treatment of subjects who received ChAdOx1 nCoV-19. Even though 175 serious adverse events (84 in the ChAdOx1 nCoV-19 group and 91 in the control group), 3 of them were correlated to challenges: transverse myelitis 14 days after booster vaccination of ChAdOx1 nCoV-19, hemolytic anemia in a control recipient, and a fever higher than 40°C in a participant who was still disguised to group assignment. Two other occurrences of transverse myelitis occurred, both of which were deemed to be unrelated to the challenge: on 10 days after the first dose of ChAdOx1 nCoV-19, which was a result of multiple sclerosis (MS), and one after 68 days of immunization in the control group. The cases of transverse myelitis instances forced the trial to be put on hold for a while, but all of the participants are either fully recovered or are in the process of recovering [85].

As for Moderna COVID-19 immunization should be administered in two doses (each 100 g, 0.5 ml) in the muscles at one-month intervals (4 weeks). The ACIP released a provisional recommendation for the usage of the Moderna COVID-19 vaccination for 18 years old people and up to contain COVID-19 on December 19, 2020. In Phase III clinical research, about 30,000 patients aged 18 to 95 years (m = 52 years) were enrolled. According to early outcomes of this clinical trial, the efficacy of the Moderna COVID-19 vaccine after two doses were 94.1 percent (95 percent confidence interval = 89.3 percent – 96.8 percent) in the prevention of symptoms, laboratory-tested COVID-19 among persons without proof of previous SARS-CoV-2 infection. It was based on data from participants who had a median follow-up of two months. Efficacy was high (86%) across all age, gender, racial, and ethnicity categories, as well as among patients with other medical conditions. COVID-19 resulted in ten hospitalizations, with nine in the placebo group and one in the vaccine group. The Moderna COVID-19 vaccine, according to preliminary findings, may give some defense against asymptomatic infection with SARS-CoV-2. Reactogenicity symptoms and signs, defined as a site for local injection or systemic adverse responses within one week of vaccination, were widespread among vaccine users, though usually mild to moderate. Systemic adverse effects were more numerous and severe in those aged 18–64 than in people aged 65 after the second dose in comparison to the first. The bulk of local and systemic adverse effects occurred within the 1–2 days of immunization, and they lasted on average 2–3 days. It was more common to have severe local or systemic adverse effects (grade 3 reactions) [84, 86].

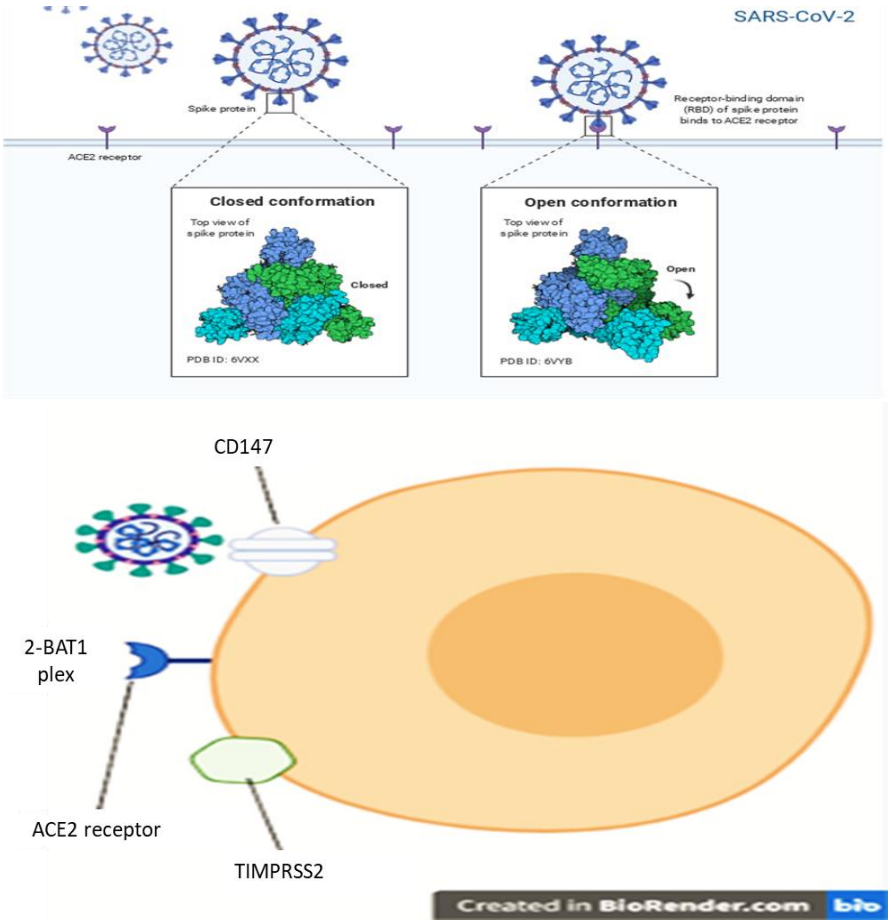

Figure 1. General structure of SARS-CoV-2 and infection mechanism of human cells. a) Glycoprotein spikes on SARS-CoV-2, b) the receptor of angiotensin-converting enzyme 2 (ACE2) protein on human cells, c) the CD147 receptor on human cells. A protein monomer spike (S) structure reveals the major molecular domains participating in pathogenesis. SARS-CoV-2 attachment and input t receptor and co-receptor of host cell involve preparing by TMPRSS2 (transmembrane serine protease 2), that splits the S protein into two parts (S1 and S2), allowing the S protein to be primed into S1 and S2 portions. S1 targets and binds the ACE2 receptor, followed by endocytosis facilitated by the virion receptor into the cell of the host. This diagram created via BioRender (https://app.biorender.com/). The spike glycoprotein structure was adopted from RCSB PDB 6VXX, as stated by Zhang *et al*. [87].

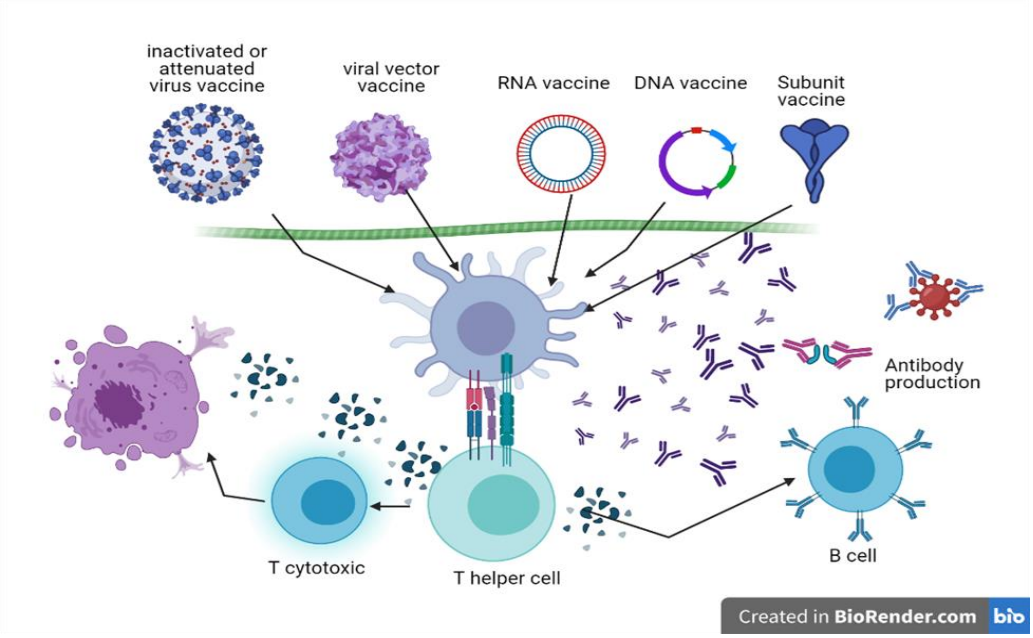

Figure 2. Strategies for Vaccine of COVID-19. Inactivated PiCoVacc is a candidate for virus vaccine of the inactivated type that encloses a purified inactivated SARS-CoV-2 virus by β-propiolactone or attenuated vaccine. Viral vector

vaccines e.g.ChAdOx1-nCov19 & Ad5-nCoV are typically genetically modified (defective replication) adenoviruses that once within the host can produce SARS-CoV-2 spike S protein. Sequences of viral peptides can be assembled into prospective epitopes with potential immune response initiation abilities to be utilized as a SARS-CoV-2 subunit vaccine. RNA-constructed vaccines such as mRNA-1273 and BNT162 contain encoding mRNA for the spike S protein of SARS-CoV-2. These are also embedded within the lipid vesicle. The DNA-constructed vaccine, e.g.INO-4800, expresses variants of SARS-CoV-2 spike protein that are frequently introduced by electroporation into cells. When the vaccine is within the host, the virus or proteins interpreted by the viral genome are engulfed by APCs (antigen-presenting cells) like dendritic cells and macrophages. Th (T helper) cells that further activate B cells and cytotoxic T cells are presented with the SARS CoV-2 expressed viral polypeptide on the surface of APCs (Tc). B cells secrete viral S- protein-specific antibodies that further neutralize virions and other viral proteins. To kill virus-infected host cells, Tc cells base an immune reaction of cytolytic type. The manufacture of memory B and T cells will supplementarily promote immunity to the host. The diagram was created using BioRender (https://app.biorender.com/).

Table 1. Some of the COVID-19 vaccination candidates that applied for clinical trials and some already in use

Vaccine	Produced company	Platform	Mode of action	Clinical trial
mRNA-1273	Moderna	mRNA vaccine Lipid nanoparticle (LNP)-encapsulated mRNA vaccine	Induces helper T cell immune responses	Phase I
BNT162	Pfizer and BioNTech	Synthetic strand of mRNA	Prepared to produce long term IgG as well as stimulation of T cell responses	Phase I/ II
CVnCoV Vaccine (CV07050101)	Curevac	Synthetic strand of mRNA	Induces neutralizing antibody responses	Phase I
INO-4800	Inovio Pharmaceuticals	Plasmid DNA vaccine which encodes for SARS-CoV-2 spike proteins	Stimulates immune responses both cellular and humoral	Phase I/ II
Sinopharm	Both Sinopharm and the Wuhan Institute of Virology	Inactivated SARS-CoV -2 virus	Excrete Immunoglobulins targeting the virus	Phase I/ II
Sinovac	Institute of Medical Biology, Chinese Academy of Medical Sciences	Formalin-inactivated and alum-adjuvanted	Produce mainly antibodies against the virus	Phase Ib/IIb
Ad5-nCoV	CanSino Bio	Viral vectored, Non-replicating adenovirus type 5 that expresses SARS-CoV-2 spike protein	Stimulate humoral immunity	Phase I
ChAdOx1 nCoV- 19 (AZD1222)	University of Oxford	Viral vectored, Non-replicating chimpanzee adenovirus that delivers the RNA into the cells	Stimulate the immune response to produce spike-specific IgG and T cell responses	Phase I/ II
Gam-COVID-Vac (Sputnik V)	Gamaleya Research Institute of Epidemiology and Microbiology, Health Ministry of the Russian Federation	Viral vectored human adeno-vector virus (rAd5 and rAd26) encoding for spike protein of SARS-CoV-2	Stimulate T cell cellular immune response	Phase I/ II
COVID-19 S-Trimer	GlaxoSmithKline and Clover Biopharmaceuticals	Recombinant subunit vaccine of S-Trimer protein of the COVID-19	Stimulate humoral immunity	Phase I
KBP-COVID-19	Kentucky BioProcessing, Inc.	Protein subunit vaccine encodes for SARS- CoV2 RBD	Stimulate humoral immunity	Phase I/ II
COVAX19	Vaxine Pty Ltd and Central Adelaide Local Health Network Incorporated	Protein subunit vaccine encodes for recombinant spike protein of COVID19 with Advax-SM adjuvant	Stimulates both humoral and cellular response	Phase I

Conclusion

Presently, SARS-CoV-2 is the focus of extensive scientific research counting vaccine initiatives, the introduction of antiviral drug trials, the appearance of new diagnostic tests, and the development of new biomarkers. The question is very clear for future research: how will the experience we have gained over the past few years help us to eliminate this pandemic and be

more prepared for future uncertainties? This review gives a summary of almost all significant vaccines for COVID-19. Some of them are now being used for vaccination, and some did not make it through the trial phase. We tried to explain the platform technology in each one of them and the advantage and disadvantages of most of them **(Figure 2 and Table 1)**. Care must be taken in following the standard operating procedures with regards to the pandemic, such as social distancing, wearing of masks, handwashing, and various other quarantine measures.

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