

REVIEW ARTICLE



Nanoarchitectonics: role of nanomaterials in vaccination strategies for curbing SARs-CoV-2/COVID-19

Iqra Zulfiqar¹, Abdul Wahab², Muhammad Usama Saeed¹, Nazim Hussain¹, Muhammad Farooq Sabar¹, Muhammad Bilal^{3,*}, and Hafiz M.N. Iqbal^{4,*} 

¹Center for Applied Molecular Biology (CAMB), University of the Punjab, Lahore, Pakistan.

²School of Agriculture Sciences and Veterinary Medicine, University of Padua, Italy.

³School of Life Science and Food Engineering, Huaiyin Institute of Technology, Huaian 223003, China.

⁴Tecnologico de Monterrey, School of Engineering and Sciences, Monterrey, 64849, Mexico.

*Corresponding authors emails: bilaluaf@hotmail.com (M. Bilal); hafiz.iqbal@tec.mx (H.M.N. Iqbal).

© The Authors 2022

ABSTRACT

With the exponential rise in infections by CoV-2 and the scarcity of antiviral therapeutics, the development of an effective vaccine for the SARS CoV-2 is critical. The emerging pandemic has prompted the international science community to seek answers in therapeutic agents, including vaccines, to battle the SARS CoV-2. The various scientific literature on SARS CoV, to a lesser degree, MERS (Middle East Respiratory Syndrome), has mentored vaccine techniques for the unique Coronavirus. This disease, COVID-19, is triggered by SARS-CoV-2 Virus that causes COVID-19 that needs vaccine protection. Vaccines producing significant amounts of virus-neutralizing antibodies with high affinity may be the only way to combat infection while avoiding negative consequences. There is a summary of numerous vaccine contenders in the review, including nucleotide, vector-based vaccines, & subunit, and attenuated & killed types. That has previously shown preventive effects against the MERS-CoV & SARS-CoV, while suggesting that these candidates may yield a safe and efficient vaccine for SARS-CoV-2. Vector-based vaccines, monoclonal antibodies, genetic vaccines, and protein subunit types for passive immunization are among the vaccination platforms currently being evaluated for the CoV-2 Virus; each has its own set of benefits and drawbacks. The clinical safety and effectiveness evidence is the main challenging research task for this possible vaccine developed in the lab. The most challenging aspect of production is constructing and validating distribution platforms worthy of mass-producing the vaccine on a larger scale. Since target vaccine groups include high-risk people above the age of 60, including severe co-morbid diseases, the healthcare staff, and those engaged in vital industries, an effective COVID-19 vaccine would need a careful confirmation of effectiveness and detrimental reactivity. The study summarises efforts devoted to developing an efficient vaccine for the new Coronavirus that devastated the global economy, people's health, and even their lives.

ARTICLE HISTORY

Received: 16-01-2022

Revised: 27-04-2022

Accepted: 1-05-2022

KEYWORDS

Nano cues;
Fabrication;
Vaccine development;
Passive immunization;
Vaccination;
COVID treatment;
Monoclonal antibody

Introduction

Coronavirus disease has been a significant concern globally [1]. In December 2019, it was first identified in the Chinese city of Wuhan and announced as a pandemic by WHO on 11 March 2020 [2]. It is caused by SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2). Deaths by SARS-COVID-19 continue to rise in most European countries [3]. Coronavirus is the single-stranded RNA (positive-sense) virus and belongs to the family Coronaviridae [4]. In the past few years, many attempts have been made to produce vaccines for CoV viruses for humans. However, the extent of the cross-protection provided via such vaccines owing to the wide sequence variation is a primary limiting factor. Many Vaccines, drug alternatives, and immunotherapeutic have also been investigated in response to the latest challenges of Ebola, Zika, & Nipah viruses, and also past CoVs, such as SARS- & MERS-CoVs [5-7].

The COVID-19 was decoded and detected in January 2020, & the genome was quickly published. The very short-term measures were taken as of mid-April to minimize SARS-CoV-2 transmission are much identical to the steps taken to decrease SARS-CoV spread during the 2002-2003 pandemic. Such short-term interventions, referred to as “tactical therapies,” have been applied occasionally. Such tactical therapies aim to control the adverse effects of the viral invasion; these treatments do not improve the immune system. EIDD 2801, favipiravir, tocilizumab, chloroquine, hydroxychloroquine, losartan, a mixture of lopinavir, and So far, the results of the tests have been a bit of a mixed bag [8]. The Virus’s incubation time varies from two to fourteen days [9]. Temperature, nausea, cough (dry), chills, breath shortness, pain in the muscle, disturbance in the stomach (mainly gastric), headache, gastric disturbances, & loss of weight are a few of the indications [10]. In chest CT scans, Lymphopenia & bilateral ground-glass opacity variations are common in certain patients. Histological analyses of biopsy extracts from the lungs revealed diffused damage with alveoli of the cellular fibro myxoid exudates on both sides. Both liver & heart specimens had some inflammatory infiltrates of interstitial mononuclear [11]. By stimulating an effective immune response, intestinal mononuclear phagocytes (MNP)

play the critical part in sustaining integrity of the epithelial barrier whilst trying to control the attack of pathogen [12]. However, a significant percentage of affected patients have no or minor signs & appear asymptomatic [13]. Individuals should be protected from contamination by social isolation, personal cleaning or hygiene, regular washing of hands, or disinfecting with hand sanitizers based on alcohol [10]. A pandemic scale that is used for the assessment of the infectious agent’s contagiousness is R_0 . Its size is determined by a variety of biological, socio-economic, and environmental variables [14]. The R_0 values vary for the SARS CoV-2 is from 2 to 3 [15], which is possibly a bit higher than the R_0 range of value for the Spanish flu, which is 0.9 to 2.1 [16].

Identifying the viral RNA by the nasopharyngeal swab or the saliva and nucleic acid test and assays that identify viral protein antigens is used to diagnose SARS-CoV-2 infection [17, 18]. The test results are just positive for the brief period in affected people on average before the 14th day after the onset of symptoms [19]. Besides that, a positive NAT (Nucleic acid testing) test result does not aid scientists in determining either the individual is immune or not [20]. As a result, serological tests are required because they can identify diverse forms of the antibodies present in the blood that can remain for months or even years. Innate immune activation, including antigen-specific T and B lymphocytes, plays a role in immune reaction to SARS CoV-2 [21]. Vaccines are urgently needed to induce defensive immune responses, mainly by a virus-neutralizing antibody directed for SARS CoV-2 [22].

COVID 19 vaccine

Vaccination is generally produced from a small quantity of weak or dead organisms that cause disease, like viruses and bacteria. Vaccines assist the body in fighting diseases rapidly and effectively by lowering the possibility of contracting the disease from real pathogens through stimulation of the natural immune response from host. The spread of the SARS CoV-2 pandemic globally has sparked tremendous progress in biotechnology, particularly vaccine development [22]. The first two vaccines were produced shortly after the structure of SARS COV-2 was released. The assembly of the Virus SARS COV-2 is made up

of an array of structural and functional proteins accompanying an RNA molecule [23]. S Protein, a P protein, E protein, and N protein are known structural proteins (nucleocapsid). Protein S helps to bind with human cell receptors, allowing viral material to fuse with the cell. The angiotensin-converting enzyme 2 (ACE2) receptor is present in significant numbers in the respiratory tract and lung parenchyma in the case of SARS COV-2 [24-27]. The bronchial ciliary epithelial cells are where SARS COV-2 first infects them [8].

Vaccination Strategies

Scientists are trying to develop effective vaccines with the least side effects. Current knowledge related to the different areas of the SARS-CoV-2-immune system shall be shown in the vaccine design, involving the vaccination routes, adjuvants, vaccine platforms, antigens selection, and dosage regimens. Virus neutralizing antibodies (nAbs) produced by infected viruses or vaccinations serve an important function to control viral infection. Single-domain antibodies nanobodies (Nbs), or the single-chain variable region fragment (scFv), monoclonal antibodies

(mAbs), and their functionally antigen-binding fragment (Fab) are now being developed for MERS-CoV and SARS-CoV [28]. In preventing or treating disease, neutralizing antibodies (nAbs) can be passively introduced into humans pre or post-viral infection (while vaccinations effectively trigger an immune response to generate nAbs specific for antigen to avoid disease). Therapeutic nAbs only last a limited time in the body, and their treatment effectiveness is determined by several criteria, including nAb titre, half-life, specificity, and total quantity. Neutralizing antibodies with minimal toxicity and strong affinity, great specificity for target protein have been utilized to treat viral infections [29]. Various strategies are involved in the Covid Vaccine. Surface exposed spike glycoprotein (S protein) is mainly targeted, producing neutralizing antibodies [30]. Vaccine for the SARS-Covid includes recombinant viral vaccines, attenuated vaccines, protein subunit vaccines, inactivated vaccines, nucleic acid vaccines or DNA vaccines. Viral vectors (both replicating and non-replicating), nanoparticles, RNA, and other materials have distinct advantages and disadvantages [31]. The COVID-19 vaccine landscape is depicted in Figure 1.

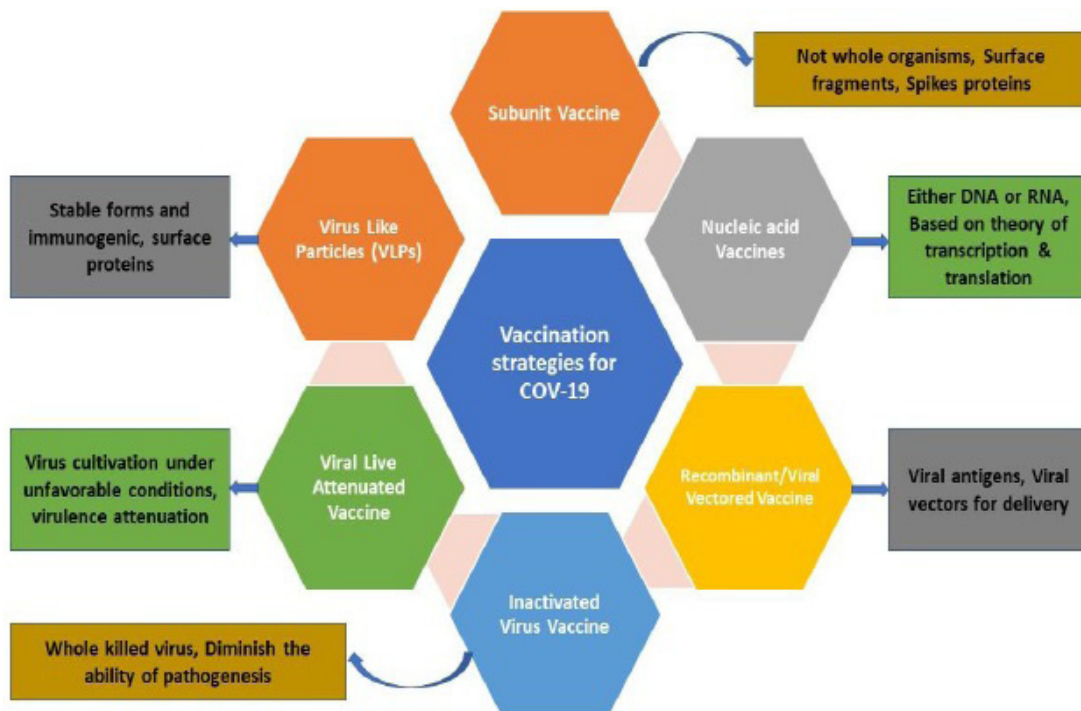


Figure 1. Illustration of various vaccination strategies for COVID-19.

Types of Vaccines against SARs-COV-2:

I. Sub-unit vaccine

Instead, whole organisms' purified antigens are used to make these vaccines, and different carriers are used to transport them. These antigens are presented by various nanoparticles, viral proteins, and peptides. These purified antigens are obtained from recombinant DNA technology or conventional processes. These antigenic determinants increase the immune system's efficiency to respond and reduce the side effects risk. Protein subunit vaccines contain mostly surface fragments of the pathogen because they are weak immunogens; protein molecules are conjugated with antigens [8]. The spike protein (S), membrane, envelope, and nucleocapsid are used as antigens, activating antibodies and producing a defensive mechanism. [33].

Subunit vaccine shows poor immunogenicity and depends on the adjuvant because it enhances the half-life of antigen and the immunomodulatory response of cytokines. The most common adjuvants used are aluminum salts, AS03, virosome, AS04, and MF59 [34]. S protein is the most suitable antigen used. S protein contains two subunits. RBM, NTD, and RBD domains are present in the S1 subunit, and S2 consists of HR 1&2, FP. Subunit vaccines have high safety levels. The most common expression system to produce recombinant proteins is the bacterial expression system. Mammalian and insect expression system is used for the antigen where posttranslational modification is needed.

- **Some examples of Subunit Vaccine are:**

- **NVX-CoV2373 (Novavax, Inc. | Emergent Bio-Solutions)**

It is a nanoparticle-based vaccine based on pre-fusion stable coronavirus S²-protein's recombinant expression. It is expressed in the Baculovirus expression system. The adjuvant company plans to use is Matrix-M to increase its immune response against spike protein [35, 36].

- **Vaccine Candidate Molecular Clamp Stabilized S protein:**

Produced via GSK and Dynavax in collaboration with Queensland University. The university used

the AS03 Adjuvant system, strengthening vaccine response and reducing the number of vaccines needed per dose. This technology has been used to produce neutralizing antibodies, which have been proved [36, 37].

- **PittCoVacc (University of Pittsburgh):**

Using a recombinant SARS-CoV-2 vaccine may be possible based on a MicroNeedle Cluster (MNA) containing the RSARS-CoV-2-S1fRS09 rSARSCoV-2 S1 (recombinant immune substances) organization. A significant increase in specific antigen antibodies was found after two weeks in mice models in the preclinical experiment. Furthermore, antibody immunogenicity was effectively conserved after gamma-ray sterilization. The statistically interesting titers that were recently reinforced considerably boost MNA-SARS-CoV-2 immunization in the initial stages [38].

Nucleic Acid Vaccine

In nucleic acid-based methods, an antigen that inscribes DNA or RNA of plasmid, such as messenger RNA, is used as a replicon of the Virus. Antigens are nucleic acids encoded proteins, causing both antibody and cell-mediated responses after being taken and released by a cell. Both methods are highly adaptable due to the ease with which they enable antigen modification. The advantage of imitating protein synthesis during infectivity is suggested by antigen generation in target cells. They allow any choice antigen distribution, regardless of whether it was extracted from bacteria, viruses, or parasites. They were allowed to produce vaccines against a wide range of pathogens. Furthermore, since the characteristics of vaccines are not based on inscribed proteins, no new development, purification, confirmation, or production services are needed for the large-scale manufacture of vaccines made from nucleic acid [33].

i. DNA Vaccines

The antigen and adjuvant are encoded in the DNA vaccine, which activates the adaptive immune system. [39]. DNA vaccines are preferred due to their stability, easy production, and simplicity. The Coronavirus genes are delivered to human cells through DNA vaccines [40]. The theory

of vaccination is based on the translocation of DNA into the cell nucleus, where antigen transcription begins and is accompanied by a translation. Plasmids are widely used as vectors in DNA vaccines. Myocytes or keratinocytes are targeted depending on the vaccine delivery method, i.e., intramuscular, subcutaneous, and intradermal. Furthermore, immature Dendritic Cells endocytose antigenic material, which is subsequently exposed to T-cells (CD4⁺ and CD8⁺) associated with MHC-2 and MHC-1 antigens on the cell surface, resulting in effective humoral and cell-mediated immune responses [39].

In the production of DNA vaccines, only those extrachromosomal plasmids having a very minimal degree of chromosomal integration are generally used. In addition, the bulk of plasmids exists at the administration facility [3]. Another benefit of this advanced technology is that plasmids that are used are simple and can be produced in more significant amounts, and the protection granted is long-lasting. Some drawbacks of DNA vaccines are that this kind of vaccine is restricted to protein immunogens [41]. Although DNA vaccines are effective in the stages before going into clinical trials, their effectiveness in the field is unconvincing, and their immunogenicity is poor than the other vaccines, including killed and live-attenuated virus vaccines [42].

➤ **INR-4800/Inovio Pharmaceuticals**

The DNA vaccine protects in the case of the SARS-CoV-2 [43]. It employs a codon-optimized S protein sequence from SARS-CoV-2 that is attached to an IgE leader sequence. XhoI and BamHI were used to synthesize and digest the IgE-spike sequence of DNA of SARS-CoV-2. Under control of IE CMV & the BGH polyadenylation signal, chymified DNA was inserted into pGX0001, an expression plasmid. The T cell reaction and functional antibodies in preclinical studies suggest that this vaccine will trigger a successful immunological response within seven days of vaccination [44].

➤ **ChAdOx1 nCoV-19**

ChAdOx1 nCoV-19 is designed to prevent MERS [45]. In this vaccine, the adenovirus vaccine vector and SARSCOV-2 spike (surface) protein

are employed. It has been changed to prevent it from replicating in human & the genetic code for processing the surface (spike) protein of COVID-19 has been inserted, enabling the adenovirus to manufacture these proteins in reaction to vaccination. Consequently, antibodies against the Spike enzyme are formed, which is located on the surface of SARSCOV-2[8].

ii. **RNA vaccines (mRNA vaccine)**

The method relies on mRNA fragments, genetic materials derived from DNA and form proteins. The vaccine is loaded with the viral mRNA, which synthesizes proteins of the current covid Virus & then is injected into the body by a modern corporation. The mRNA is processed by lymph nodes' immune cells and produces distinct antigens of viral proteins recognized by other immune cells.

mRNA is a relatively new noncontagious and non-fusing technology with a low chance of insertional mutagenesis. non-replicating RNA and virus-derived self-replicating RNAs are currently being investigated. The immunogenicity of the mRNA can be reduced, and changes can be made to improve the vaccine's stability [46]. Furthermore, since mRNA is the least immunogenic genetic vector, anti-vector immunity is prevented, allowing the vaccine to be administered several times [47].

An RNA vaccine is made in vitro using a DNA plasmid template and a recombinant RNA polymerase reaction. A poly(A) tail and synthetic cap analogue are inserted to form a mature RNA chain. Various delivery mechanisms (such as lipid nanoparticles, Nano-emulsions, and cationic peptides) or facilitated transfection methods are used further to stabilize the cells (gene gun and electroporation). Traditional mRNA vaccines work by triggering transient antigen expression in the cytoplasm of the host cells.

Self-amplifying mRNA vaccines, which contain the genes coding for the targeted antigen and the genes necessary for self-replication, are another platform (mostly RNA-dependent RNA polymerase). Delayed antigen expression can prevail in self-amplifying mRNA vaccines, limiting vaccine efficacy. However, since the self-amplifying mRNA vaccine platform achieves

higher yields, an equal level of safety can be achieved at even lower doses [48].

The main benefit of the mRNA vaccine is that it avoids the time-consuming process of purifying viral proteins, saving months to years in standardization and mass processing. As a result, vaccines of DNA and mRNA can be brought to clinical trials fast and are less complicated to design. They are an optimal target for future SARS-CoV-2 vaccine development & other associated pandemics [33]. Therefore, among the sciences of mRNA addition into the vaccine, the body causes that viral protein to behave in the same way it may have directed its host to do [8].

➤ **mRNA-1273 (Moderna TX, Inc.)**

It could be a Lipid Nanoparticle (LNP) antibody made up of processed mRNA encodes the complete-length pre-fusion, stable SARS-CoV-2 surface glycoprotein (spike) protein. It may elicit a complex antiviral response in responding to an overly S-protein. It is also considered moderately protected because it is not made up of live pathogen or inactivated pathogen subunits. For Phase II trials, the FDA has a rapid-step approval process. The organization released the Stage I combating agent information cycles of eight members with various dose thresholds. Candidates from the 25 µg set are similar to the cure sera. Members who received the 100 µg dose, on the other hand, had significantly greater nAb levels than healing sera levels. Although three individuals showed three systemic indications following the arrangement of the existing measures of 250 mg dosage levels, the vaccine was found to be highly efficacious and long-lasting in the 25 mg and 100 mg dose cohorts [49].

II. Recombinant/Viral vectored vaccine

Virus vectors, which use one “Virus’s genome to deliver another ‘virus’s antigen, are mostly used along with traditional virus vaccines, allowing the advancement of a platform technology for the virus production. This approach can generate vaccines on a broader scale [33]. Viral vector-based vaccines are developed by engineering a viral vector to carry coronavirus genes and eventually replicate in host cells. —replication of coronavirus results in the synthesis of coronavirus proteins and the activation of the immune system.

Adenoviruses, togaviruses, paramyxoviruses, rhabdoviruses, parvoviruses, and poxviruses are also potential vectors. Such viral vectors may be made to be non-replicating or replicating [3]. Viral proteins will serve as adjuvants, improving the immune response, which results in the production of many antibodies & longer immunity, and lowering antigen dosage needed [33]. The need for a legitimate confirmation of the Virus’s safety and function and a diverse set of purification techniques are all drawbacks of these kinds of vaccinations. VBV’s efficacy might be hampered by the host’s pre-existing immunity. To circumvent this, nonhuman or uncommon serotype vectors might be utilized. The potential for viral genes to incorporate into the host genome and uncontrolled replication is a major safety concern [3].

➤ **Adenovirus vectored vaccine (CanSino Biologics Inc., Beijing Institute of Biotechnology):**

The supplies of adenoviruses have many advantages, including their easy oral or nasal administration and human non-pathogenicity, especially for mutants having defective replication. Pre proof. This recombinant Ad5 (adenovirus type-5 vector) is used to express SARS-recombinant CoV-2’s spike protein. It is replication-defective. It was generated by cloning a fully optimized S Protein gene and a plasminogen signal-peptide-activator gene into the adenovirus type-5 vector without E1 and E3. This vaccine was developed with a Microbix Biosystem Admax method [33].

➤ **ChAdOx1/University of Oxford**

ChAdOx1 adenovirus recombinant vaccine is produced with S Glycoprotein optimized with the codon, synthesized at 5’ end with the leading sequence of the (tPA) plasminogen activator. The SARS-CoV-2 coding sequences were propagated in shuttle plasmid for the amino acids 2-1273 in number and tPA leaders. Containing the Tetracycline Operator sites and the Bovine Growth hormone (GGH) Polyadenylation signal among the Gateway® re-cloning site is responsible for the shuttle plasmid encoding main early human cytomegalovirus (IE CMV) genes. The Adenovirus vector genome is formed by injecting the CoV-2 S gene into the ChAdOx1 adenovirus

genome's E1 locus into the Bacterial Artificial Chromosome. It was allowed to replicate in these cell lines: T-Rex 293.

HEK and then purified by ultracentrifugation of this CsCl gradient. Improved immunity to the Virus can be seen as there is no subgenomic RNA within intra-muscularly vaccinated animals observed from preclinical testing. Previous experiments showed that the immune response is to be regulated with one single shot. Phase II clinical studies have been conducted to assess the vaccine in a specific population group [16].

➤ **LV-SMENP-DC (Shenzhen Geno-Immune Medical Institute)**

The interaction of the lentiviral vector with the conserved regions of the SARS CoV-2 auxiliary proteins and the protease employed by the SMENP minigens, resulting in a dendritic-cell (DC) architecture that is ready for LV-SMENP-DC immunization. Subcutaneous immunization of the vaccine introduces antigens on APC (antigen-presenting cells), which eventually work Cytotoxic T cells and develop a reliable and stable reaction [50].

➤ **Coroflu (University of Wisconsin-Madison FluGen Bharat Biotech)**

A self-limiting infection of flu, M2SR, is improved by adding the spike protein quality arrangement of SARS-CoV-2. Furthermore, the antibody transmits the flu infection's hemagglutinin protein, triggering a protective response against both viruses. Because it requires M2 quality, the M2SR is self-limiting and does not undergo replication. It has the ability to penetrate the cell and activate the cell's resistance to infection. It could be treated intranasally, mimicking the natural path of viral infection. Compared to the Compared to intramuscular infusions, this route enacts a few forms of the resistant framework and has larger immunogenicity [51].

III. Inactivated virus vaccine

Inactivated virus vaccines, also known as WKV (whole Killed Virus) vaccines, reflect a pathogen whose ability to infect and replicate has been stopped, rendering it sterile while maintaining its ability to act immunogen, enabling the immune

system to respond if inserted into a host. The vaccines operate by showing the same epitopes as the Virus may have posed in the absence of the vaccine, triggering an immune response. Antibodies against at least eight distinct proteins were found in the sera of infected people, indicating the existence of several moieties revealed on the pathogen's membrane [52].

The Virus grows in cell lines that form a substratum for significant amounts of antigen production. Virus propagation is also accompanied by cleansing before vaccine inactivation and concentration. In most approved human antiviral vaccines, formaldehyde and beta-propiolactone are used to inactivate the Virus. To achieve adequate effectiveness of inactivated vaccines, multiple doses are required [48]. Whole killed vaccines are healthy and capable of developing SARS-CoV neutralizing antibodies that are specific. Thus, killed vaccines could be considered a potential candidate for the SARS-CoV-2 vaccine. This vaccine form has a higher degree of stability [33]. Given many benefits, inactivated vaccines' production has limitations: bulk budding (biosafety stage 3) pathogens and complete sterilization of cultivated pathogens. Successful SARS-CoV sterilization, with the help of UV radiation, has been shown in bulk.

➤ **β-propiolactone WKV SARS-CoV (Tor-2 strain)**

β-propiolactone inactivated WKV SARS -CoV (Tor-2 strain) was a potential vaccine candidate as it induced neutralizing antibodies and was able to minimize the virus load in the pulmonary tract in the mouse model [53]. However, there is little clinical evidence of mouse models getting infected by such a disease; consequently, examining models where this strain is more potent and persistent is critical.

IV. Viral Live attenuated vaccines

These types of vaccines are generated by cultivating microbes under unfavorable environmental conditions that evaluate virulence attenuation whilst keeping the capability to activate the response of the immune system. The immune responses elicited by an attenuated live virus are similar to those elicited by a typical infection, but without the possibility of occurring

disease, and offer longer-lasting immunity, which is defensive immunity. The vaccine production shall adhere to strict guidelines, with reliability, effectiveness, and reproducibility taking precedence. These vaccines are more effective because they last longer, present the full viral antigen complement of the host's immune system, transmit antigen to proper cell and tissue compartments to create proteins produced inside the body, and help generate a robust T-cell response cytotoxic. Viral Live attenuated vaccines offer several disadvantages, including the likelihood of alterations leading to virulence reversal and contraindication in the immunocompromised people [8].

➤ **DeINS1-SARS-CoV2-RBD /University of Hong Kong**

A strain of influenza vaccine named LAV lacks the NS1 gene. It has rearranged to display CoV-2 Spike protein's RBD domain on the surface, & it is developed in MDCK (Madin Darby Canine Kidney Cells) cells and chick embryos. It may be in nasal spray and can be more immunogenic than the wild influenza virus [16].

V. Virus-like particles (VLPs)

The unique idea is to produce Vaccine virus-like particles (VLPs) and recombinant proteins. Surface proteins used to produce VLPs are commonly used to produce antiviral vaccines. VLP production in cells is a multi-stage procedure followed by regeneration into immunogenic and stable forms. VLP development for non-enveloped viruses tends to have serious problems. Vaccines based on nucleic acid are intriguing from the perspective of platform technology, as they enable the creation of multiple antigens using the same method. Both humoral and cellular immune responses are elicited [54]. These vaccines could be made using cell fermentation of *Escherichia coli* and related plasmid isolation and purification technologies that ensure structural integrity [55]. Due to the lack of genetic material, VLPs are made up of integrated viral proteins that are non-infectious. These entities have the same shape and size as real viruses and can trigger immune responses. Because VLPs do not include viral genomes (non-replicating), they are safer for elderly vaccine recipients or immune-compromised individuals.

Approximately 18 VLPs vaccines are currently in preclinical development. Novavax manufactured one of them, which is claimed to be in phase iii trials. The future applicability of VLPs is critical due to the possibility of future COVID-19 waves and the emergence of new diseases [56].

Various companies and institutions manufacture different vaccine candidates with different vaccine targets varying targets and properties are depicted in table 1.

Adoptive immunity/Passive Immunization

This is the application of the preformed antibodies for various disease treatments. That can be accomplished with polyclonal serum produced in other animals like horses, serum from convalescent patients, neutralizing monoclonal antibodies developed from humanized antibodies, or hybridoma technology [16].

I. Convalescent Plasma therapy

Up till now, no particular therapy for COVID-19 has been successful. During outbreaks, CP therapy (convalescent plasma therapy) has been accepted as an effective therapy [60]. This is regarded as the quintessential immunotherapy, having previously been used to cure and deter infectious diseases like MERS, SARS, the H1N1 pandemic, mumps, and measles [61]. Neutralizing immune globulins from the Convalescent Plasma therapy can overcome viremia, prevent new contamination, or enhance the removal of the infected cells, which could explain the effectiveness of such classic adoptive immunotherapy. Several trials evaluating the therapeutic effects of CP have conclusively demonstrated that administering neutralizing antibodies to seriously ill patients improved their health condition in all the patients without causing any deaths. The amount of dosage for CP therapy has not yet been prescribed, and Randomized Clinical Trials are needed not just to rule out the effects of many other medications but also to assess the safety & effectiveness of the CP therapy. [62]. Medical effects (e.g., standardization of the body temperature, pulmonary lesions absorption, ARDS resolution, stopping the use of artificial ventilators, etc.) and mortality rates were significantly different in the CP patient community [16].

Manufacturer	Candidate	Vaccine Targets	Platform	Vaccine Properties	Clinical Phase
Novavax	NVX-CoV2373	S protein (complete length)	Protein subunit	Matrix M adjuvanted complete length recombinant SARS-CoV-2 glycoprotein nanoparticle vaccine	Phase I/II NCT04368988 Phase II NCT04533399 Phase III 2020-004123-16 NCT04611802
Bharat Biotech International Limited	BBV152	Complete Virus	Inactivated	Whole inactivated virion	Phase I/II CTRI/2020/07/026300 CTRI/2020/09/027674 NCT04471519 Phase III CTRI/2020/11/028976 NCT04641481
AstraZeneca/ University of Oxford	ChAdOx1 nCoV-19/ AZD1222/ Covishield	S protein (complete length)	Non-replicating viral vector	Adenovirus from chimpanzee that contains the SARS-CoV-2 surface glycoprotein genomic sequence	Phase I PACTR202005681895696 Phase I/II PACTR202006922165132 Phase II/III NCT04400838 Phase III ISRCTN89951424
Sinopharm/ Beijing institute of biological products	Inactivated SARS-CoV-2 vaccine	Complete Virus	Inactivated		Phase I/II ChiCTR2000032459 Phase III NCT04560881
NIAID/ Moderna	mRNA-1273	Complete length S protein with two proline substitutions (K986P and V987P)	RNA	mRNA encoding the surface spike protein was encapsulated in lipid nanoparticles	Phase I NCT04283461 Phase II NCT04405076 Phase II/III NCT04649151 Phase III NCT04470427
Janssen Pharmaceutical Companies	Ad26.COV2.S	Two mutations at the furin cleavage site (R682S and R685G) and two proline substitutions (K986P and V987P) in complete-length S protein	Non-replicating viral vector	Ad26 vector	Phase I NCT04509947 Phase I/II NCT04436276 Phase II EUCTR2020-002584-63-DE Phase III NCT04505722
Health Ministry of the Russian Federation/ Gamaleya Research Institute	Sputnik V	S protein (complete length)	Non-replicating viral vector	Recombinant adenovirus type 5 (rAd5) and type 26 (rAd26) carrying gene for surface glycoprotein of SARS-CoV-2 (rAd5-S and rAd26-S)	Phase I/II NCT04436471 Phase III NCT04530396

Table 1. Different candidate vaccines are in the third stage of clinical testing [57-59].

II. Monoclonal Antibody

Monoclonal antibodies (mAbs), also known as therapeutic antibodies, are copies of a distinctive parent that may bind to a single epitope — for example, they contain monovalent affinity [63]. Direct infusion of monoclonal antibodies (mAbs) can be an essential tool for CoV regulation in people who have been exposed to the Virus. Patients suffering from SARS have been shown to have strong neutralizing antibody responses. Use of such a group of monoclonal antibodies (mAbs) that attack the MERS-CoV Spike protein particular domains. The Six distinct epitope groups that interact with the membrane fusion, receptor binding, and sialic acid-binding sites, such mAbs, bind to these epitopes, marking MERS-CoV S protein's three primary entry functions [64].

By targeting numerous epitopes of S protein & their tasks, these antibodies can pose a potential approach to increasing humoral defence towards emerging CoVs. The ability of the CoV RBD-specific neutralizing mAbs to cross-neutralize relies heavily on the resemblance of RBDs. Antibodies against CoV RBD will also neutralize SARS-like (SL) CoVs [65]. The RBD of the S protein of the CoV-2 has been shown to bind with CoV-2 specific human mAbs CR3022, suggesting a possible therapeutic agent that could be used alone or in combination with other therapies to treat the COVID-19 [64]. The combinatorial influence of mAbs identifying various epitopes on the Virus's surface can be called for virus neutralization to attain greater efficiency in disease treatment and prevention. This may appear to be more efficient and avoid viral escape.

For COVID-19, a combination of drug remdesivir and mAbs may be an optimal treatment choice. Before proving the effectiveness of this combination treatment, further research is needed. Humanized Nanobodies (VH/VHH, sdAB, single domain antibodies) or completely human antibodies (Hu-scFvs) can penetrate through the membrane of cells infected with viruses (trans bodies) as well as bind to or interact with the biological activities of the proteins of replicating Virus, resulting in prevention of virus replication. Thus, trans bodies for CoV proteins inside the cells like proteases [3CLpro (the cysteine-like), PLpro (papain-like), and many non-structural proteins (nsps)] that are crucial as well as for the

CoV transcription process and replication process can be produced for healthy, non-immunogenic, widely adequate passive immunization of the CoV infected subjects as well as treatment of infected individuals [30]. In terms of efficacy, specificity, lower chance of blood-borne infection, purity, and other considerations, the use of mAbs to cure and prevent viral diseases can solve some of the disadvantages of CP therapy. A broad range of monoclonal antibodies (mAbs) has also been developed for anti-platelet, anti-tumor, and antiviral treatment [16].

To fight COVID-19, neutralizing monoclonal antibodies (mAbs) are being produced by targeting the receptor-binding domain (RBD) of SARS-CoV-2 spike (S) protein. Anti-RBD mAbs block the S protein's attachment to its corresponding receptor, angiotensin-converting enzyme 2 (ACE2), on the target cells of a host. For the treatment of COVID-19, three neutralising mAb regimens have been awarded emergency use authorization [66]. Casirivimab & imdevimab attach to different epitopes on RBD, with the dissociation constant KD values of 46 & 47 pM. Imdevimab attaches to the RBD of S protein on the front or bottom left side, whereas casirivimab binds to the spike-like loop on the top (overlapping with the ACE2-binding site) [67, 68]. (2) Bamlanivimab engages an epitope on RBD in either its open & closed conformations, with a dissociation constant (KD) of 71pM, spanning 7 of roughly 25 side chains found to make interaction with ACE2. [69]. (3) Bamlanivimab & etesevimab attach to separate but overlapping epitopes inside RBD of the SARS CoV-2's protein. Etesevimab interacts with RBD conformation (up/active) with a dissociation constant (KD) of 6.45 nM [69]. It has the LALA variation in Fc region, which causes it to have no effector activity.

Challenges during the formulation of the SARs-COVID-19 vaccine

The sudden spread of the coronavirus disease (COVID-19), triggered by SARS-CoV-2 Coronavirus, has resulted in a vast global epidemic and is a significant national crisis [70, 71]. The current severe infection crisis has emphasized a need for more innovative therapeutic and prevention solutions to minimize the disease's dangers and spread. Despite the world's dire need for a preventive vaccine to effectively combat the

outbreak, this kind of vaccine's invention faces significant obstacles [71].

➤ Evaluation of vaccine efficacy

The ability of COVID-19 vaccines to produce neutralizing and binding antibodies is the widespread criterion used to test them. However, various vaccine assessment approaches and models allow it impossible to analyze different vaccine effectiveness [72]. Vaccines boost viral immunity, but they may also induce inflammation. This can appear as inflammation or pain at the injection site and as primary symptoms like fever or exhaustion (symptoms like COVID-19 infection). Many vaccination reactions affect just a limited number of individuals and are slight. In humans or mice, the development of antibodies by a vaccine often does not imply that perhaps the vaccine can protect against disease [73].

The effectiveness of a human vaccine is established through a series of trials related to those used to verify safety. If the vaccine activates the response of immune system, like antibodies detection in the phase I trial, the broader stage II plus stage III trials will be made to evaluate whether this vaccine protects against contagious diseases. Restricted human trials could also be conducted to determine the vaccine's dosage and timetable. Some vaccines produce a robust immune response following only one injection, while some other vaccines need a booster shot, a month or more later. The technique used also lengthens study phase. To see how a vaccine avoids COVID 19 infection in patients who have been exposed to it, it can be studied in stage III trials in the environment where the viral infection is still present. Harmful factors in the vaccine effectiveness include purity and safety of adjuvant, information deficits regarding respective contributions of adaptive immune responses, innate immune responses to defense against particular pathogens, and the exact mechanism of action of single adjuvants.

Anti-SARS COV 2 vaccine effectiveness risk factors include


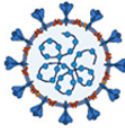
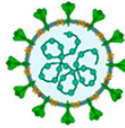
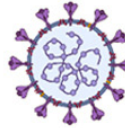
Worldwide immune deficiency, a risk factor against anti-COVID-19 vaccine effectiveness, specifically in elders, is prone to various factors that lead to an immune system weakness. Obesity-related/obesity disorders, like metabolic

syndrome, diabetes (type II), and immune-mediated cancers are also caused by these causes. Antigen detection defects reduced immune cell amount and efficiency, enhanced length/level and duration of the humoral immune modifications of components, impaired cellular response activation, & memory cell abnormalities are some of the mechanisms behind these diseases. Other causes contributing to immunodeficiency include age-related improvements in immune and humoral cells, malnutrition, immunosenescence, (19) telomere shortening, and protein-energy micronutrient deficit [74]. Furthermore, previous or current therapies have an impact on vaccine scalability, especially in immunocompromised individuals (in both adults [75] & children [76, 77]). Due to elevated concentrations of IL-6 [78] and reduced IgG levels, the world's current obesity incidence in adults & children is also a contributing factor towards the ineffectiveness of Vaccine for SARS-CoV-2. Parasitic and respiratory infections include severe pneumonia, impairing the immune reaction toward the anti-SARS CoV-2 Vaccine. Figure 2 shows a schematic representation of an epidemiological comparison of respiratory viral infections [79].

Routes of Delivery: Another critical consideration that determines vaccine effectiveness is the route of drug administration. Parenteral and Mucosal vaccination are two popular vaccination methods. The subcutaneous (SC), intramuscular (IM), and intradermal (ID) routes are the most common parenteral routes [80]. The ID application starts a more significant adaptive immune response than the IM application due to improved DC penetration of the dermis, resulting in substantial dosage sparing effect. On the other hand, improved efficacy is linked to a lower safety profile [81]. Mucosal vaccines, which can be given intranasally or orally, have many benefits, including eliminating the need for a needle and a reduced risk of severe side effects.

Evaluation of vaccine safety

Besides the tissue damage inflicted by COVID-19, like kidney and lung damage, the intensity of the disease and high death rates in COVID-19 patients are linked to underlying chronic conditions like hypertension, chronic obstructive pulmonary disease, and cardiovascular disease [82].

Epidemiological Comparison of Respiratory Viral Infections				
Disease	Flu (Common cold)	COVID-19	SARS	MERS
Disease Causing Pathogen	 Influenza virus	 SARS-CoV-2	 SARS-CoV	 MERS-CoV
R_0 Basic Reproductive Number	1.3	2.0 - 2.5 *	3	0.3 - 0.8
CFR Case Fatality Rate	0.05 - 0.1%	~3.4% *	9.6 - 11%	34.4%
Incubation Time	1 - 4 days	4 - 14 days *	2 - 7 days	6 days
Hospitalization Rate	2%	~19% *	Most cases	Most cases
Community Attack Rate	10 - 20%	30 - 40% *	10 - 60%	4 - 13%
Annual Infected (global)	~ 1 billion	N/A (ongoing)	8098 (in 2003)	420
Annual Infected (US)	10 - 45 million	N/A (ongoing)	8 (in 2003)	2 (in 2014)
Annual Deaths (US)	10,000 - 61,000	N/A (ongoing)	None (since 2003)	None (since 2014)

* COVID-19 data as of March 2020.

Figure 2. A schematic representation of an epidemiological comparison of respiratory viral infections. It was reprinted from Ref. [79] with permission under the Creative Commons Attribution 4.0 International License. The Figure was created with the “BioRender.com” template and exported under the terms of the premium subscription.

Surprisingly, diabetes and COVID19 have a bidirectional association [83]. Diabetes, mainly, on the other hand, is linked to a higher threat of COVID-19 infection.

Some scientists say SARS-CoV can cause islet damage and severe diabetes mellitus (insulin-dependent) [84]. If there is a correlation between the progression of diabetes and vaccination with certain types of vaccinations—mainly killed and live attenuated, that contains the most constituents of the killed Virus, it remains unanswered and needs further research. As a result, high clinical vaccine safety trials and other epidemiologic studies about vaccinations and diabetes risk are required to evaluate and track the safety of the COVID-19 vaccines. Research lab animal models are used to research and develop safe and reliable vaccines. Such animal models should mimic the progression of the disease in humans. Owing to the differences between humans and rodents, normal inbred mice strains are not vulnerable to COVID-19 infection. ACE2 receptors are a type of receptor found in the body (Anon, 2020D). There is a growth requirement for the transgenic

mice that express this hACE2 receptor. For SARS-CoV, two animal models were earlier created (primate Macaques model and hACE2 transgenic mice model). However, the present situation necessitates consistent reproduction & distribution of such models of animals to fulfil the requirements of scientists worldwide [85].

Manufacturing of Vaccine

Tens of millions of shots are necessary for pandemic vaccines. If the manufacturing lines are still in place, this phase would require a minimum of six months. All new Vaccines necessitate a new manufacturing process with many quality controls measures. The vaccine producer must guarantee that each batch is of a consistent standard, which requires frequent testing. Furthermore, since vaccine manufacture is a biological operation, specific vaccine batches will eventually fail for unknown reasons, causing further delays in development. Only a few companies in the world are capable of mass-producing vaccines on a broad scale to satisfy this pandemic’s demands [8].

In the event of a pandemic outbreak, such as COVID-19, the regular timeframe for vaccine research, manufacture, as well as distribution must be hastened in order to stop the disease's spread. Biopharmaceutical companies responding to the demand would require considerable financial resources and an accelerated FDA (Food and Drug Administration) clearance process for vaccination medicines. To help such businesses, the United States government launched Operation Warp Speed, a public-private collaboration (OWS). OWS promotes vaccine mass manufacturing as well as underwrites financial commitments that help firms to increase vaccine development without having to wait for the full findings of Phase 3 clinical study. Via this programme, vaccine producers have secured billions of dollars in financing to help vaccines scale-up [69].

The most excellent alternative for addressing the COVID-19 vaccine production issues, including speed, effectiveness, plus safety, has evolved as a platform-based vaccine. The elements that make up the platform. Modularity is supported by platform-based vaccines such as the A "base carrier," like lipid nanoparticles and viral vectors can be used in vaccinations. "Modules" are the elements that are inserted into the base carrier. The modules can be of mRNA and DNA strands that are custom-made for the target illness and could be encased in a base carrier to produce the appropriate vaccination [86]. Once a system has been authorized for a vaccine, it will be possible to make more vaccines using the same system in the future, allowing for faster development, large-scale manufacture, and regulatory approval. To tackle COVID-19, there are primarily two types of platform-based vaccinations in use: viral vector vaccines & mRNA vaccines [87].

Regulation of Vaccines

Before commencing every stage of the human testing technique, the developer should demonstrate that the vaccine exhibits early signals of safety and effectiveness for individuals screened. Research ethics commissions review clinical study programs. Regulatory agencies such as the FDA (Food and Drug Administration) and EMA (the European Medicines Agency) monitor the whole vaccine production process unless approved for widespread use. These evaluations typically take several days or months to complete

[88]. While those approvals may be accelerated in the case of a pandemic, several future COVID-19 vaccinations utilize emerging technology, so authorities will not be willing to depend on previous experience of identical vaccines to speed things up. The COVID-19 vaccine's developers were granted a 12–18-month deadline, although vaccine development has traditionally taken 15–20 years. Various biomedical research associations are collaborating on producing the Vaccine of COVID-19, employing various processes. Since most vaccines that go through clinical trials fail due to safety and effectiveness issues, it's critical to get many people involved in the race [89].

Discussion

The study addresses methods for formulating COVID-19 vaccine and difficulties in vaccine production. Coronavirus is most common type of Virus in the Coronaviridae family and the order Nidovirales [90, 91]. These are also distinguished by the spike structures on their surface, which are club-shaped. COVID-19 is mainly transmitted by respiratory particles/droplets, but direct social interaction and fecal to the oral transmission can also be involved [92]. COVID-19 mostly leads to respiratory tract problems causing symptoms such as nausea, fever, common cold, nasal inflammation, diarrhea, dry cough, and sore throat, which escalate to severe pneumonia, breathing difficulties, and patient mortality. These viruses are enveloped with a segmented, single-stranded RNA genome of around 30 kb. Positive sense, the genome is the largest among all RNA viruses [93]. 'CoVs' genetic material is especially susceptible to recombination, contributing to the development of new strains with modified virulence [33].

Live vaccines are yet to be tested in the human trials for COVID-19. Previous research has demonstrated that, as opposed to the first vaccine dose, booster vaccination by live-attenuated Virus produces only a tiny immune response [94, 95]. Furthermore, past COVID-19 infection-induced immunity may reduce the effectiveness of the live attenuated vaccine, and the existence of neutralizing antibodies may be linked to virus neutralization. Furthermore, genome instability can result in virulence reversion, especially viruses with a high mutation rate [96-98]. As a result, live vaccines might not be the best choice for

preventing COVID-19 disease [99]. Like killed and recombinant subunit vaccinations, other traditional vaccination treatments are being analyzed in clinical studies against COVID-19 infection. Their effectiveness is constrained by short-term immune memory and lower response rates. As a result, all methods necessitate the use of potent adjuvants. A significant barrier to killed vaccines is the chance of a negative impact associated with increased virus-mediated illness and lethal effects. 89]. New vaccine variants like messenger RNA vaccines & recombinant vaccines are also thoroughly researched to overcome these hurdles. The FDA & EMA recently approved using mRNA vaccine candidates, both BNT162 & mRNA-1273. Moreover, other candidates for a vaccine, such as Ad26. COV2.S, including ChAdOx1-SARS-CoV-2, are being considered [3].

The formulation and production of the vaccine is an important matter, but it will most certainly take several months to address. To be sure, innovative vaccine methods pose several safety questions. However, techniques based on better production practices guidelines and adequate preclinical and clinical research conducted under the supervision of regulatory authorities should maintain an excellent safety rating [100]. While several firms have stated that the Vaccine for COVID-19 will be available soon, it will be challenging to achieve in reality [45]. The most significant factor is that vaccine should be effective equally in the short and that vaccine should be effective equally in the short and long term prior to it being released to the public. To prevent dangerous conditions, anything that would be performed on humans must be first reviewed for purity, and afterwards, sterile manufacturing lines should be established [101].

The second justification is that vaccines must be both safe and reliable. As a result, for any potential Covid vaccine, both of these factors should be considered, and primary vaccination errors should be reduced by changing the doses/quantity of intake. Nucleic acid vaccines work by inserting their nucleic acids into each vaccinated cell, causing them to produce immunogenic proteins [102]. While some recent literature seems to be promising, the efficacy of these theories in humans is doubtful. The attenuated vaccines will be SARS CoV-2 mutants that have been genetically modified to be fewer or may not be pathogenic at all. They are still most immunogenic, although

there is this possibility that mutations will cause them to turn pathogenic [103]. Killed viruses, synthetic peptides, and viral fragments are immunogenic to lower degrees. Since coronavirus immunity has many uncertainties, developing a vaccine is complicated. Future SARS COV-2 mutations may arise at any moment, putting every vaccine in question. The top candidates for the vaccine are already being delivered to a small group of people, with high hopes of reducing the increase of COVID-19.

Conclusion

SARS-CoV-2 became a prominent concern since it was announced as a disease outbreak, & it has caused global economic events to be halted. Scientists worldwide are collaborating to reuse drugs, create vaccines, or introduce devices to slow the spread of such a devastating pandemic. The COVID-19 candidates for vaccines based on different platforms have been reported in significant numbers. For managing the lethal COVID-19, scientists are looking for the appropriate vaccine candidates or drugs/medicines that are both effective & appropriate. Vaccine production is a lengthy process that includes many phases, like preclinical testing & clinical development; it is a three-phase method. As a result of this new Coronavirus, scientists have been pushed to use experimental methods to speed up the vaccine production process. "Vaccine must have an extremely favorable benefit-risk contour; with higher efficiency, only minor or intermittent side effects, with no severe ailments", according to WHO. This vaccine should be adequate for people of all ages, including pregnant & lactating mothers, also should ensure the accelerated onset of defense with only a single injection. It should also protect a minimum of one year after administration. Using new technology in vaccine production necessitates rigorous research to ensure the vaccine's safety and effectiveness. Numerous preclinical experiments have demonstrated that the Spike protein is a crucial viral antigen in producing vaccines. While research is ongoing to enhance COVID-19 prevention, cure, and management, published clinical data for various CoV treatment interventions is limited. The best choice for increasing the proportion of the population immune to SARS-CoV-2 is to use vaccines that induce neutralizing antibodies on a broad scale.

There is no proper treatment for severe COVID 19i, but social distancing is both socially and economically quite expensive. As a result, developing an appropriate vaccine and attempting to incorporate immune-enhancing innovative therapies, including shorter-term initiatives to find a tactical repurposed cure, must be significant community health targets. We assume to obtain the critical method for preventing infection as quickly as possible. While it is unknown if an effective SARS-CoV-2 vaccine will be created, the scientific community's efforts to do so are unparalleled. As a consequence, COVID 19 vaccinations are a never-ending story.

Acknowledgements

Consejo Nacional de Ciencia y Tecnología (CONACYT) is thankfully acknowledged for partially supporting this work under Sistema Nacional de Investigadores (SNI) program awarded to Hafiz M.N. Iqbal (CVU: 735340).

Competing interests

The author(s) declare no conflicting interests.

References

- Weston, S. and M. Frieman, *COVID-19: knowns, unknowns, and questions. mSphere* 5: e00203-20. 2020.
- Hiscott, J., et al., *The global impact of the coronavirus pandemic. Cytokine & growth factor reviews*, 2020. **53**: p. 1-9.
- Strizova, Z., et al., *Principles and Challenges in anti-COVID-19 Vaccine Development. International Archives of Allergy and Immunology*, 2021: p. 1-11.
- Gorbalenya, A.E., et al., *Severe acute respiratory syndrome-related Coronavirus: The species and its viruses—a statement of the Coronavirus Study Group. 2020.*
- Munjal, A., et al., *Advances in developing therapies to combat Zika virus: current knowledge and future perspectives. Frontiers in microbiology*, 2017. **8**: p. 1469.
- Dhama, K., et al., *Advances in designing and developing vaccines, drugs, and therapies to counter Ebola virus. Frontiers in immunology*, 2018. **9**: p. 1803.
- Sheahan, T.P., et al., *Comparative therapeutic efficacy of remdesivir and combination lopinavir, ritonavir, and interferon beta against MERS-CoV. Nature communications*, 2020. **11**(1): p. 1-14.
- Calina, D., et al., *Towards effective COVID-19 vaccines: Updates, perspectives and challenges. International journal of molecular medicine*, 2020. **46**(1): p. 3-16.
- Lauer, S.A., et al., *The incubation period of coronavirus disease 2019 (COVID-19) from publicly reported confirmed cases: estimation and application. Annals of internal medicine*, 2020. **172**(9): p. 577-582.
- Control, C.f.D. and Prevention, *Coronavirus Disease 2019: COVID-19. 2020.*
- Xu, Z., et al., *Pathological findings of COVID-19 associated with acute respiratory distress syndrome. The Lancet respiratory medicine*, 2020. **8**(4): p. 420-422.
- Caër, C. and M.J. Wick, *Human intestinal mononuclear phagocytes in health and inflammatory bowel disease. Frontiers in Immunology*, 2020. **11**: p. 410.
- Shang, W., et al., *The outbreak of SARS-CoV-2 pneumonia calls for viral vaccines. npj Vaccines*, 2020. **5**(1): p. 1-3.
- Delamater, P.L., et al., *Complexity of the basic reproduction number (R0). Emerging infectious diseases*, 2019. **25**(1): p. 1.
- Park, S.E., *Epidemiology, virology, and clinical features of severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2; Coronavirus Disease-19). Clinical and experimental pediatrics*, 2020. **63**(4): p. 119.
- Kaur, S.P. and V. Gupta, *COVID-19 Vaccine: A comprehensive status report. Virus research*, 2020: p. 198114.
- Cheng, V.C., et al., *Escalating infection control response to the rapidly evolving epidemiology of the coronavirus disease 2019 (COVID-19) due to SARS-CoV-2 in Hong Kong. Infection Control & Hospital Epidemiology*, 2020. **41**(5): p. 493-498.
- Weissleder, R., et al., *COVID-19 diagnostics in context. Science translational medicine*, 2020. **12**(546).
- Tan, W., et al., *Viral kinetics and antibody responses in patients with COVID-19. MedRxiv*, 2020.
- Jiang, C., et al., *Molecular detection of SARS-CoV-2 being challenged by virus*

- variation and asymptomatic infection. *Journal of Pharmaceutical Analysis*, 2021. **11**(3): p. 257-264.
21. Thevarajan, I., et al., *Breadth of concomitant immune responses prior to patient recovery: a case report of non-severe COVID-19*. *Nature medicine*, 2020. **26**(4): p. 453-455.
 22. Speiser, D.E. and M.F. Bachmann, *COVID-19: Mechanisms of vaccination and immunity*. *Vaccines*, 2020. **8**(3): p. 404.
 23. Yang, P. and X. Wang, *COVID-19: a new challenge for human beings*. *Cellular & molecular immunology*, 2020. **17**(5): p. 555-557.
 24. Hoffmann, M., et al., *SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor*. *cell*, 2020. **181**(2): p. 271-280. e8.
 25. Wang, Q., et al., *Structural and functional basis of SARS-CoV-2 entry by using human ACE2*. *Cell*, 2020. **181**(4): p. 894-904. e9.
 26. Li, Y., et al., *Physiological and pathological regulation of ACE2, the SARS-CoV-2 receptor*. *Pharmacological research*, 2020: p. 104833.
 27. Lukassen, S., et al., *SARS-CoV-2 receptor ACE 2 and TMPRSS 2 are primarily expressed in bronchial transient secretory cells*. *The EMBO journal*, 2020. **39**(10): p. e105114.
 28. Jiang, S., C. Hillyer, and L. Du, *Neutralizing antibodies against SARS-CoV-2 and other human coronaviruses*. *Trends in immunology*, 2020. **41**(5): p. 355-359.
 29. Jiang, S., et al., *Neutralizing antibodies for the treatment of COVID-19*. *Nature biomedical engineering*, 2020. **4**(12): p. 1134-1139.
 30. Dhama, K., et al., *COVID-19, an emerging coronavirus infection: advances and prospects in designing and developing vaccines, immunotherapeutics, and therapeutics*. *Human vaccines & immunotherapeutics*, 2020. **16**(6): p. 1232-1238.
 31. Wang, N., J. Shang, and S. Jiang, *Lanying Du*. Subunit vaccines against emerging pathogenic human coronaviruses. *Front Microbiol*, 2020. **11**: p. 298.
 32. Mahrosh, H.S. and G. Mustafa, *The COVID-19 puzzle: a global nightmare*. *Environment, Development and Sustainability*, 2021: p. 1-28.
 33. Pandey, S.C., et al., *Vaccination strategies to combat novel corona virus SARS-CoV-2*. *Life sciences*, 2020: p. 117956.
 34. Mohan, T., P. Verma, and D.N. Rao, *Novel adjuvants & delivery vehicles for vaccines development: a road ahead*. *The Indian journal of medical research*, 2013. **138**(5): p. 779.
 35. Coleman, C.M., et al., *Purified coronavirus spike protein nanoparticles induce Coronavirus neutralizing antibodies in mice*. *Vaccine*, 2014. **32**(26): p. 3169-3174.
 36. Tu, Y.-F., et al., *A review of SARS-CoV-2 and the ongoing clinical trials*. *International journal of molecular sciences*, 2020. **21**(7): p. 2657.
 37. Lee, J., *These 23 companies are working on coronavirus treatments or vaccines—'here's where things stand*. *Market watch*, 2020.
 38. Kim, E., et al., *Microneedle array delivered recombinant coronavirus vaccines: Immunogenicity and rapid translational development*. *EBioMedicine*, 2020. **55**: p. 102743.
 39. Hobernik, D. and M. Bros, *DNA vaccines—how far from clinical use?* *International journal of molecular sciences*, 2018. **19**(11): p. 3605.
 40. Pandey, S.C., et al., *Vaccination strategies to combat novel corona virus SARS-CoV-2*. *Life sciences*, 2020. **256**: p. 117956.
 41. Ferraro, B., et al., *Clinical applications of DNA vaccines: current progress*. *Clinical infectious diseases*, 2011. **53**(3): p. 296-302.
 42. Silveira, M.M., G.M.S.G. Moreira, and M. Mendonça, *DNA vaccines against COVID-19: Perspectives and challenges*. *Life sciences*, 2021. **267**: p. 118919.
 43. Safety, T., *Immunogenicity of INO-4800 for COVID-19 in Healthy Volunteers*. *ClinicalTrials.gov*, 2020.
 44. Smith, T.R., et al., *Immunogenicity of a DNA vaccine candidate for COVID-19*. *Nature communications*, 2020. **11**(1): p. 1-13.
 45. Le, T.T., et al., *The COVID-19 vaccine development landscape*. *Nat Rev Drug Discov*, 2020. **19**(5): p. 305-306.
 46. Zhang, C., et al., *Advances in mRNA vaccines for infectious diseases*. *Frontiers in Immunology*, 2019. **10**: p. 594.

47. Mulligan, M.J., et al., *Phase 1/2 study to describe the safety and immunogenicity of a COVID-19 RNA vaccine candidate (BNT162b1) in adults 18 to 55 years of age: interim report*. MedRxiv, 2020.
48. Vogel, A.B., et al., *Self-amplifying RNA vaccines give equivalent protection against influenza to mRNA vaccines but at much lower doses*. Molecular Therapy, 2018. **26**(2): p. 446-455.
49. Data, M.A.P.I.B., *Against SARS-CoV-2 Variants of Concern* <https://investors.modernatx.com/news-releases/news-release-details/moderna-announces-positive-initial-booster-data-against-sars-cov>. Accessed on May, 2021. **6**.
50. Ullah, S., et al., *A review of the progress of COVID-19 vaccine development*. Duzce Medical Journal, 2021. **23**(Special Issue): p. 1-23.
51. UW–Madison, F., *Bharat Biotech to develop CoroFlu, a coronavirus vaccine*. 2020.
52. Guo, J.-P., et al., *SARS corona virus peptides recognized by antibodies in the sera of convalescent cases*. Virology, 2004. **324**(2): p. 251-256.
53. See, R.H., et al., *Comparative evaluation of two severe acute respiratory syndrome (SARS) vaccine candidates in mice challenged with SARS coronavirus*. Journal of general virology, 2006. **87**(3): p. 641-650.
54. Sarkar, B., et al., *Virus like particles-A recent advancement in vaccine development*. The Microbiological Society of Korea, 2019. **55**(4): p. 327-343.
55. Huang, X., et al., *Escherichia coli-derived virus-like particles in vaccine development*. npj Vaccines, 2017. **2**(1): p. 1-9.
56. Balkrishna, A., et al., *Nanotechnology Interventions in the Management of COVID-19: Prevention, Diagnosis and Virus-Like Particle Vaccines*. Vaccines, 2021. **9**(10): p. 1129.
57. Organization, W.H., *World Health Organization DRAFT Landscape of COVID-19 Candidate Vaccines—14 July 2020*.
58. Dai, L. and G.F. Gao, *Viral targets for vaccines against COVID-19*. Nature Reviews Immunology, 2021. **21**(2): p. 73-82.
59. Mellet, J. and M.S. Pepper, *A COVID-19 vaccine: big strides come with big challenges*. Vaccines, 2021. **9**(1): p. 39.
60. Organization, W.H., *Use of convalescent whole blood or plasma collected from patients recovered from Ebola virus disease for transfusion, as an empirical treatment during outbreaks: interim guidance for national health authorities and blood transfusion services*. 2014, World Health Organization.
61. Duan, K., et al., *Effectiveness of convalescent plasma therapy in severe COVID-19 patients*. Proceedings of the National Academy of Sciences, 2020. **117**(17): p. 9490-9496.
62. Zhang, B., et al., *Treatment with convalescent plasma for critically ill patients with severe acute respiratory syndrome coronavirus 2 infection*. Chest, 2020. **158**(1): p. e9-e13.
63. Ngandu, T., et al., *A 2 year multidomain intervention of diet, exercise, cognitive training, and vascular risk monitoring versus control to prevent cognitive decline in at-risk elderly people (FINGER): a randomised controlled trial*. The Lancet, 2015. **385**(9984): p. 2255-2263.
64. Zhang, W., et al., *Molecular and serological investigation of 2019-nCoV infected patients: implication of multiple shedding routes*. Emerging microbes & infections, 2020. **9**(1): p. 386-389.
65. Zeng, L.-P., et al., *Cross-neutralization of SARS coronavirus-specific antibodies against bat SARS-like coronaviruses*. Science China Life Sciences, 2017. **60**(12): p. 1399-1402.
66. Taylor, P.C., et al., *Neutralizing monoclonal antibodies for treatment of COVID-19*. Nature Reviews Immunology, 2021. **21**(6): p. 382-393.
67. Ashwathi, P., N. Venkateswaramurthy, and R.S. Kumar, *The New Outlook of Monoclonal Antibodies in Neutralizing Target Cells in COVID-19*. Journal of Drug Delivery and Therapeutics, 2021. **11**(5-S): p. 138-142.
68. Hansen, J., et al., *Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail*. Science, 2020. **369**(6506): p. 1010-1014.
69. Hirshberg, J.S., et al., *Monoclonal antibody treatment of symptomatic COVID-19 in pregnancy: initial report*. American Journal of Obstetrics & Gynecology, 2021. **225**(6): p. 688-689.

70. Organization, W.H., *World Health Organization coronavirus disease (COVID-19) dashboard*. 2020.
71. Organization, W.H., *International clinical trials registry platform (ICTRP)*. <http://www.who.int/ictrp/en/>, 2010.
72. Chandrashekar, A., et al., *SARS-CoV-2 infection protects against rechallenge in rhesus macaques*. *Science*, 2020. **369**(6505): p. 812-817.
73. Wojda, T.R., et al., *The Ebola outbreak of 2014-2015: From coordinated multilateral action to effective disease containment, vaccine development, and beyond*. *Journal of global infectious diseases*, 2015. **7**(4): p. 127.
74. Tsoukalas, D., et al., *Association of nutraceutical supplements with longer telomere length*. *International journal of molecular medicine*, 2019. **44**(1): p. 218-226.
75. Ventura, M.T., et al., *Immunosenescence in aging: between immune cells depletion and cytokines up-regulation*. *Clinical and Molecular Allergy*, 2017. **15**(1): p. 1-8.
76. Savy, M., et al., *Landscape analysis of interactions between nutrition and vaccine responses in children*. *The Journal of nutrition*, 2009. **139**(11): p. 2154S-2218S.
77. Arvas, A., *Vaccination in patients with immunosuppression*. *Turkish Archives of Pediatrics/Türk Pediatri Arşivi*, 2014. **49**(3): p. 181.
78. Eliakim, A., et al., *Reduced tetanus antibody titers in overweight children*. *Autoimmunity*, 2006. **39**(2): p. 137-141.
79. Bilal, M., Khan, M. I., Nazir, M. S., Ahmed, I., & Iqbal, H. M. *Coronaviruses and COVID-19—complications and lessons learned for the future*. *J Pure Appl Microbiol*, (2020). **14**(1): 725-731.
80. Zhou, G. and Q. Zhao, *Perspectives on therapeutic neutralizing antibodies against the Novel Coronavirus SARS-CoV-2*. *International journal of biological sciences*, 2020. **16**(10): p. 1718.
81. Kikkert, M., *Innate immune evasion by human respiratory RNA viruses*. *Journal of innate immunity*, 2020. **12**(1): p. 4-20.
82. Yang, J., et al., *Prevalence of comorbidities and its effects in patients infected with SARS-CoV-2: a systematic review and meta-analysis*. *International Journal of Infectious Diseases*, 2020. **94**: p. 91-95.
83. Rubino, F., et al., *New-onset diabetes in Covid-19*. *New England Journal of Medicine*, 2020. **383**(8): p. 789-790.
84. Yang, J.-K., et al., *Binding of SARS coronavirus to its receptor damages islets and causes acute diabetes*. *Acta diabetologica*, 2010. **47**(3): p. 193-199.
85. Bao, L., et al., *The pathogenicity of SARS-CoV-2 in hACE2 transgenic mice*. *Nature*, 2020. **583**(7818): p. 830-833.
86. Hume, H.K.C. and L.H. Lua, *Platform technologies for modern vaccine manufacturing*. *Vaccine*, 2017. **35**(35): p. 4480-4485.
87. Slaoui, M. and M. Hepburn, *Developing safe and effective Covid vaccines—Operation Warp ‘Speed’s strategy and approach*. *New England Journal of Medicine*, 2020. **383**(18): p. 1701-1703.
88. Grenham, A. and T. Villafana, *Vaccine development and trials in low and lower-middle income countries: Key issues, advances and future opportunities*. *Human vaccines & immunotherapeutics*, 2017. **13**(9): p. 2192-2199.
89. Mazur, N.I., et al., *The respiratory syncytial virus vaccine landscape: lessons from the graveyard and promising candidates*. *The Lancet Infectious diseases*, 2018. **18**(10): p. e295-e311.
90. Kahn, J.S. and K. McIntosh, *History and recent advances in coronavirus discovery*. *The Pediatric infectious disease journal*, 2005. **24**(11): p. S223-S227.
91. Fehr, A.R. and S. Perlman, *Coronaviruses: an overview of their replication and pathogenesis*. *Coronaviruses*, 2015: p. 1-23.
92. Guan, W.-j., et al., *Clinical characteristics of 2019 novel coronavirus infection in China*. *MedRxiv*, 2020.
93. Belouzard, S., et al., *Mechanisms of coronavirus cell entry mediated by the viral spike protein*. *Viruses*, 2012. **4**(6): p. 1011-1033.
94. Christenson, B. and M. Böttiger, *Measles antibody: comparison of long-term vaccination titres, early vaccination titres and naturally acquired immunity to and booster effects on the measles virus*. *Vaccine*, 1994. **12**(2): p. 129-133.
95. Kongsgaard, M., et al., *Adaptive immune responses to booster vaccination*

- against yellow fever virus are much reduced compared to those after primary vaccination. *Scientific reports*, 2017. **7**(1): p. 1-14.
96. Hanley, K.A., *The double-edged sword: How evolution can make or break a live-attenuated virus vaccine*. *Evolution: Education and Outreach*, 2011. **4**(4): p. 635-643.
97. Luring, A.S., J.O. Jones, and R. Andino, *Rationalizing the development of live attenuated virus vaccines*. *Nature biotechnology*, 2010. **28**(6): p. 573-579.
98. Minor, P.D., *Live attenuated vaccines: Historical successes and current challenges*. *Virology*, 2015. **479**: p. 379-392.
99. Mok, D.Z. and K.R. Chan, *The effects of pre-existing antibodies on live-attenuated viral vaccines*. *Viruses*, 2020. **12**(5): p. 520.
100. Milstien, J., et al., *Reaching international GMP standards for vaccine production: challenges for developing countries*. *Expert review of Vaccines*, 2009. **8**(5): p. 559-566.
101. Tetro, J.A., *Is COVID-19 receiving ADE from other coronaviruses?* *Microbes and infection*, 2020. **22**(2): p. 72-73.
102. Leitner, W.W., H. Ying, and N.P. Restifo, *DNA and RNA-based vaccines: principles, progress and prospects*. *Vaccine*, 1999. **18**(9-10): p. 765-777.
103. Perlman, S. and J. Netland, *Coronaviruses post-SARS: update on replication and pathogenesis*. *Nature reviews microbiology*, 2009. **7**(6): p. 439-450.



Publisher's note: Eurasia Academic Publishing Group (EAPG) remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial 4.0 International (CC BY-NC 4.0) licence, which permits copy and redistribute the material in any medium or format for any purpose, even commercially. The licensor cannot revoke these freedoms as long as you follow the licence terms. Under the following terms you must give appropriate credit, provide a link to the licence, and indicate if changes were made. You may do so in any reasonable manner, but not in any way that suggests the licensor endorsed you or your use. If you remix, transform, or build upon the material, you may not distribute the modified material.

To view a copy of this licence, visit <https://creativecommons.org/licenses/by-nc/4.0/>.