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WAYS OF MAKING EFFECTIVE AND SAFE VACCINES AGAINST SARS-CoV -2

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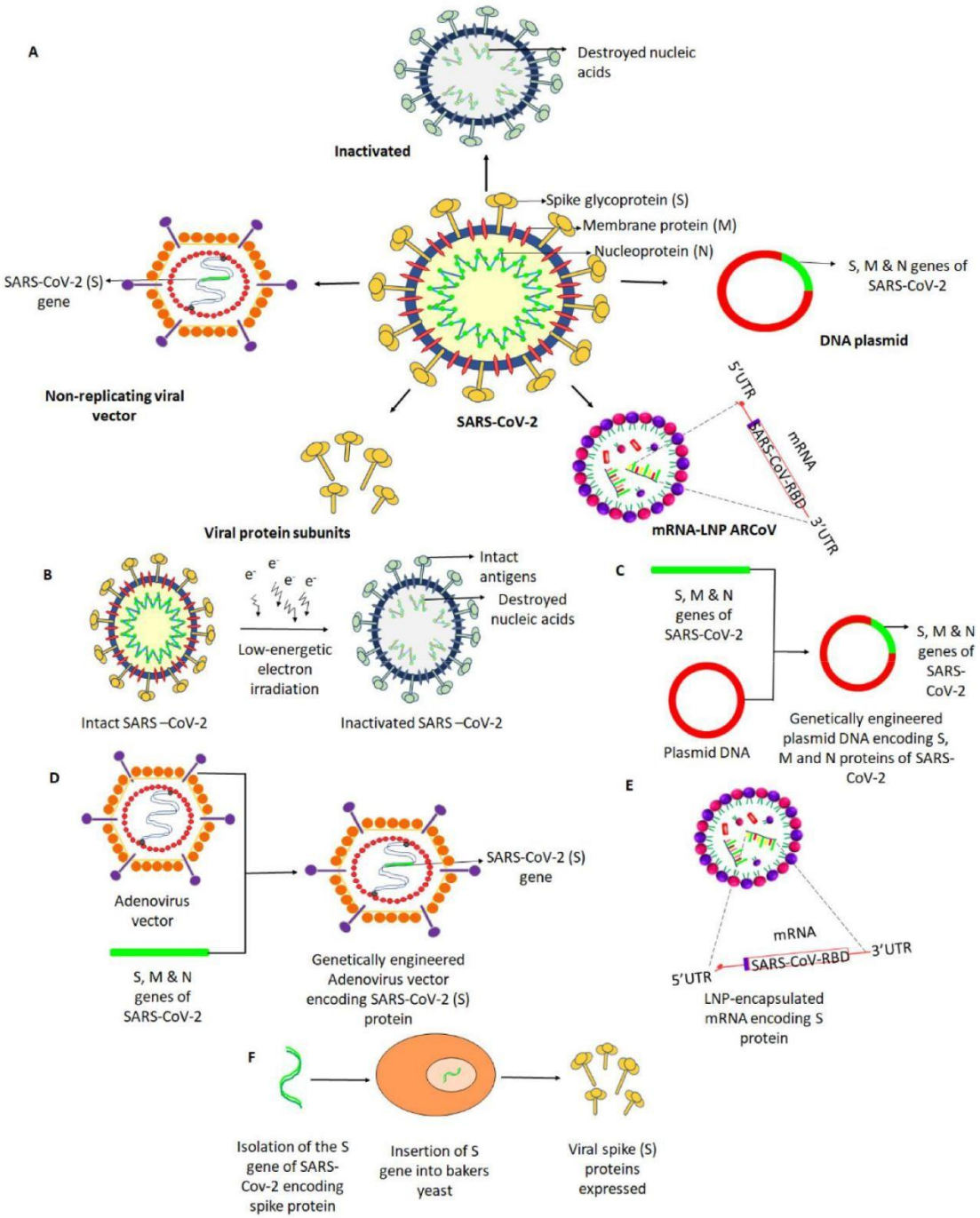
ABSTRACT
Severe acute respiratory syndrome (SARS) is an emerging infectious disease caused by SARS–associated coronavirus (SARS-CoV). Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) is another new type of coronavirus that causes the Coronavirus Disease 2019 (COVID-19), which has been the most challenging pandemic in this century. SARS-CoV-2 which is genetically similar to SARS-CoV and Middle East respiratory syndrome coronavirus (MERS-CoV) is an enveloped, single and positive-stranded RNA virus. Considering its high mortality and rapid spread, an effective vaccine is urgently needed to control this pandemic. Vaccines, such as inactivated vaccines, attenuated vaccines, nucleic acid-based vaccines, and vector vaccines, which have already been demonstrated their prophylactic efficacy against MERS-CoV and SARS-CoV, so these candidates could be used as a potential tool for the development of a safe and effective vaccine against SARS-CoV-2. The inactivated SARS-CoV vaccine may be the first one available for clinical use because it is easy to generate; however, safety is the main concern. The spike (S) protein of SARS-CoV is the major inducer of neutralizing antibodies, and the receptor-binding domain (RBD) in the S1 subunit of S protein contains multiple conformational neutralizing epitopes. This suggests that recombinant proteins containing RBD and vectors encoding the RBD sequence can be used to develop safe and effective SARS-CoV-2 vaccines.

KEYWORDS: Corona virus; Pandemic; SARS-CoV-2; COVID-19; MERS-CoV; Vaccine.

1. INTRODUCTION
Coronaviruses (CoVs) are a group of related viruses that can cause respiratory tract infection in humans ranging from mild symptoms to lethal outcomes. In the past 18 years, three novel coronaviruses have crossed the species barrier to infect humans and cause human-to-human transmission. The first lethal coronavirus SARS-CoV emerged in 2002 in Guangdong Province, China. During the 2002–2004 outbreak, SARS-CoV had infected 8,098 people and resulted in 774 SARS-associated deaths (~ 10% mortality rate) across 29 countries before it disappeared. Despite efforts from the scientific community, no vaccine became commercially available (WHO, 2003). In September 2012, the world experienced the emergence of the Middle East respiratory syndrome (MERS) coronavirus, originated in Saudi Arabia. The disease has affected 27 countries, resulting in 2494 cases and 858 deaths (~ 35% mortality rate). MERS cases are still being reported but no major outbreak has been declared since 2015. As in the case of SARS, no commercial vaccine is available for MERS (WHO, 2020).

Explanations behind the absence of commercial and effective vaccines for SARS and MERS are fluctuated. On account of MERS, almost certainly, the vaccine development was postponed due to the shortage of reasonable and cost-effective small animal models during pre-clinical experimentation. Also, it is likely that a vaccine has not been conveyed on account of the low interest in putting resources into a vaccine for a disease that has delivered moderately low and geographically concentrated cases. This last factor may have additionally added to the absence of a vaccine for SARS, as in it was viewed as inconsequential to keep putting resources into a vaccine for an infection whose cases stopped to be accounted for in 2004.

Graphical Abstract



Coronavirus disease 2019 (COVID-19) is a current pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The first cases were reported from Wuhan, China, in December 2019 (Del Rio et al., 2020). Currently there is no specific antiviral drug against SARS-CoV-2, finding a vaccine for this virus therefore remains a high priority. Vaccines are the most effective and economical means to prevent and control infectious diseases. The development of an effective vaccine against SARS-CoV-2 infection is urgently required. Although no vaccines are commercially available for SARS and MERS, past and current vaccine development efforts against these diseases might be of high value for the development of an effective vaccine for COVID-19. So far, many pharmaceutical companies and academic institutions worldwide have launched their programs on vaccine development against SARS-CoV-2.

2. THE GENETICS OF SARS-COV

Coronaviruses are the largest group of RNA viruses from the subfamily Orthocoronavirinae or Coronavirinae in the family Coronaviridae, in the order Nidovirales. They are enveloped viruses with a positive-sense single-stranded RNA genome and a nucleocapsid of helical symmetry (Cherry et al., 2017). They have characteristic

club-shaped spikes that project from their surface, which in electron micrographs create an image reminiscent of the solar corona, from which their name derives (Almeida JD et al., 1968). Their genome size is relatively large for RNA viruses, between 27 and 34 kB (de Groot et al., 2011). Coronaviruses infect mammals and birds causing varied symptoms such as respiratory tract disease and diarrhea. In humans, coronavirus infections have been shown to be potentially lethal. Until now, there are seven genera of CoVs that are known to infect humans. Four of these genera, including Human Coronavirus 229E (HCoV-229E), Human Coronavirus OC43 (HCoV-OC43), Human Coronavirus NL63 (HCoVNL63), and Human Coronavirus HKU1 (HCoV-HKU1) have been identified as causing up to a third of community-acquired upper respiratory tract infections. The other three CoVs, Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), Middle East Respiratory Syndrome Coronavirus (MERSCoV), and Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), are highly pathogenic and can lead to severe respiratory diseases and fatal outcome in infected patients (CDC, 2020).

SARS-CoV-2 is a positive-strand RNA virus that belongs to the group of Betacoronaviruses. The genome of SARS-CoV-2 is approximately 29,700 nucleotides long and shares 79.5% sequence identity with SARS-CoV and 50% with MERS-CoV (Lu R. et al., 2020). The six functional open reading frames (ORFs) are arranged in order from 5′ to 3′: replicase (ORF1a/ORF1b), spike (S), envelope (E), membrane (M) and nucleocapsid (N) (Figure 1). In addition, seven putative ORFs encoding accessory proteins are interspersed between the structural genes (Chan J. F. et al., 2020). Most of the proteins encoded by SARS-CoV-2 have a similar length to the corresponding proteins in SARS-CoV. Of the four structural genes, SARS-CoV-2 shares more than 90% amino acid identity with SARS-CoV except for the S gene, which diverges (Lu R. et al., 2020). The replicase gene covers two thirds of the 5′ genome, and encodes a large polyprotein (pp1ab), which is proteolytically cleaved into 16 non-structural proteins that are involved in transcription and virus replication. Most of these SARS-CoV-2 non-structural proteins have greater than 85% amino acid sequence identity with SARS-CoV (Chan J. F. et al., 2020).

The SARS-CoV–like virus that exists in animals does not cause typical SARS-like disease in the natural hosts and is not transmitted from animals to humans. Under certain conditions, the virus may have evolved into the early human SARS-CoV, with the ability to be transmitted from animals to humans or even from humans to humans, resulting in localized or even global outbreaks and mild to severe human disease. An early report by Zhou et al. identified a closely related SARSr-CoV genome sequence, RaTG13, which shared a 96% whole-genome sequence identity with SARS-CoV-2, indicating a probable bat origin of SARS-CoV-2 (Zhou et al., 2020). Since then, more SARS-CoV-2-related viral genome sequences from bats have been reported from Eastern China and Japan, and from pangolins in China. However, the immediate animal ancestor or progenitor virus, the equivalent of the >99% identical SARS-CoV sequences identified in civets during the SARS outbreak in 2003, remains elusive for SARS-CoV-2. Identification of the origin and immediate progenitor viruses are not only important academically, but also critical for public health measures to prevent future outbreaks caused by SARS-CoV-2 or closely related viruses.

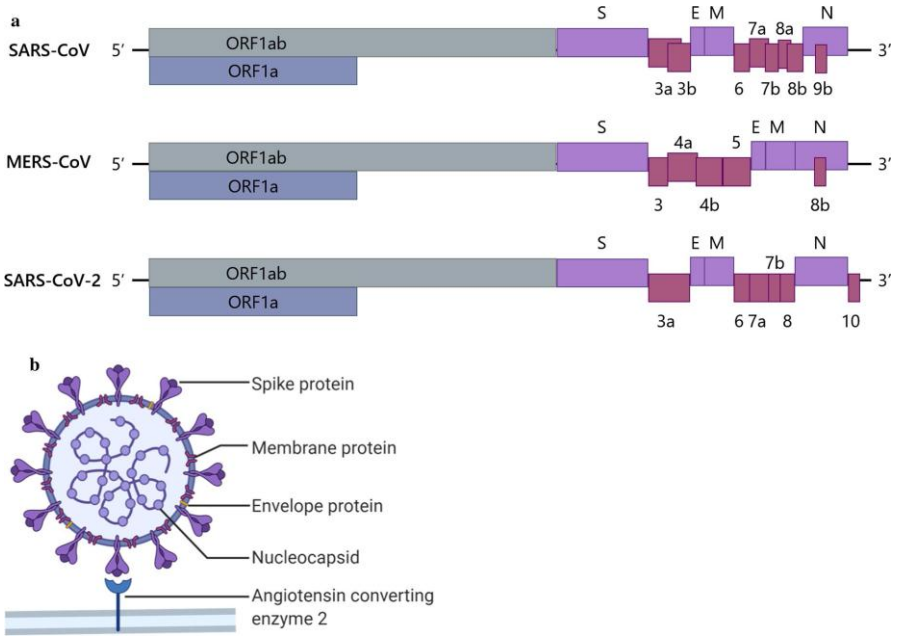


Figure 1: The genome and virion structure of coronaviruses (CoVs). a) The genome structure of SARS-CoV, MERS-CoV, and SARS-CoV-2. b) The virion structure of SARS-CoV.

3. ANTIGEN SELECTION FOR SARS-COV VACCINES

Many viral proteins are essential for the life cycle of CoVs. For entering target cells, S protein first binds to cellular receptors through its receptor-binding domain (RBD), and the receptor-virus complex is subsequently translocated to endosomes (Figure 2) (Du L et al., 2009). Both SARS-CoV and SARS-CoV-2 S proteins bind to angiotensin- converting enzyme 2 (ACE2), while the S protein of MERS-CoV uses dipeptidyl peptidase-4 (DPP4) as its cellular receptor (Wang N et al., 2020). At the endosome, S protein is further cleaved into S1 (RBD-containing) and S2 (non-RBD-containing) subunits, and the S2 subunit mediates fusion between the viral envelope and the host cell membrane (Du L et al., 2009). After entering the cell, several Nsps, particularly RNA-dependent RNA polymerase (Nsp12) and helicase (Nsp13), mediate the replication of the CoV genome and the transcription of CoV mRNA (Snijder EJ et al., 2016). The CoV mRNA is further translated into different nonstructural and structural proteins. The N proteins bind to CoV genomic RNA to form viral nucleocapsids, and S, E, M proteins form the envelope of CoV. After assembly, viral particles bud through an endoplasmic reticulum (ER)-Golgi pathway and exit the cells by exocytosis (Du L et al., 2009).

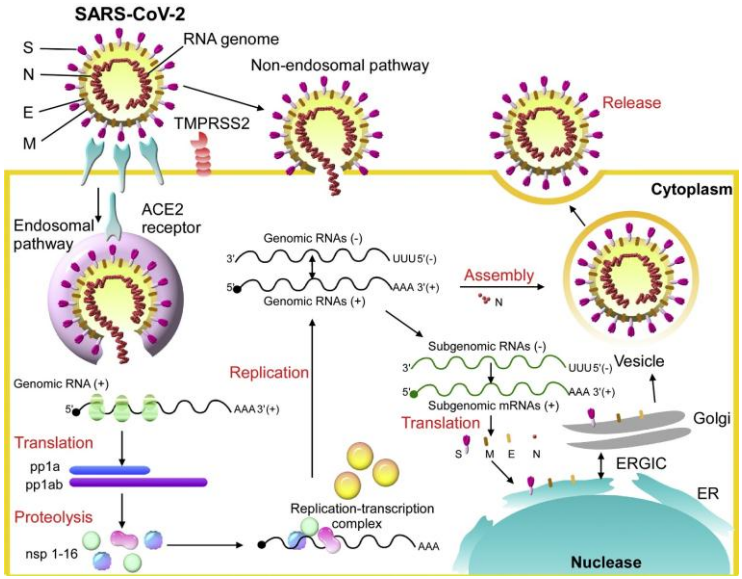


Figure 2: The putative life cycle of SARS-CoV-2.

3.1 Spike protein (S protein)

The S protein of coronaviruses (CoVs) is responsible for virus binding, fusion, and entry and is a major inducer of neutralizing antibodies. S protein is currently the most promising antigen formulation for SARS-CoV-2 vaccine research. First, it is surface exposure and thus is able to be directly recognized by host immune system. Second, it mediates the interaction with host cell by binding to the receptor ACE2, which is essential for subsequent virus entry to target cells and causing subsequent pathogenicity (Wrapp D et al., 2020). Finally, the homologue proteins were already used for vaccine development against SARS-CoV and MERS-CoV, and were proved to be effective (Zhou Y et al., 2018; Du L et al., 2009). Studies have shown that antibodies generated against the S protein are long-lasting and immunodominant in recovered SARS patients. In addition, the anti-S antibody can neutralize SARS-CoV and MERS-CoV and provides protective effects in animals and humans. Moreover, many S protein-based vaccines against SARS-CoV and MERS-CoV have been shown to elicit potent immune responses and protective effects in preclinical models (Coleman CM et al., 2014; Muthumani K et al., 2015). These results corroborate that CoV S protein serves as an ideal vaccine target to induce neutralizing antibodies and protective immunity.

The monomer of S protein from SARS-CoV-2 contains 1273 amino acids, with a molecular weight of about 140 kDa. Self-association naturally assembles the S protein into a homo-trimer, typically similar to the first class of membrane fusion protein (Class I viral fusion protein). The S protein contains two subunits (S1 and S2) (Figure 3). The S1 subunit can be further defined with two domains termed the N-terminal domain (NTD) and the C-terminal domain (CTD). The receptor binding domain (RBD) is located in the CTD. S2 subunit contains the basic elements required for membrane fusion, including an internal membrane fusion peptide (FP), two 7-peptide repeats (HR), a membrane proximal external region (MPER), and a trans-membrane domain (TM) (Li F, 2016). Recently, the structure of the SARS-CoV-2 S trimer in the pre-fusion conformation and the RBD domain in complex with ACE2 has been successfully determined, which has provided valuable information for vaccine design based on this protein (Wrapp D et al., 2020). So far, the potential fragments of S protein for use as antigens in vaccine development include the full-length S protein, the RBD domain, the S1 subunit, NTD, and FP.

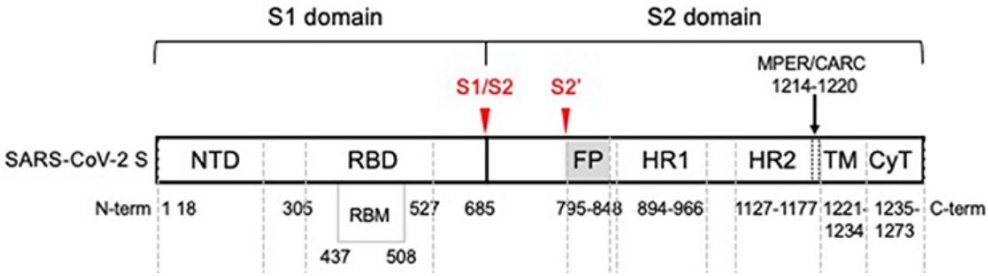


Figure 3: Strategies for designing vaccines for severe acute respiratory syndrome (SARS) using spike (S) protein.

i. The full-length S protein

Full-length proteins are likely to maintain the correct conformation of the protein, capable of providing more epitopes and exhibiting higher immunogenicity. Muthumani et al. reported that DNA vaccine encoding MERS-CoV S protein was immunogenic in mice, camels, and rhesus macaques. Animals immunized with the DNA vaccine show reduced typical clinical symptoms including pneumonia during the infection (Muthumani et al., 2015).

ii. RBD

RBD, a fragment (193 aa residues) in the middle of S1 subunit of S protein, is responsible for virus binding to the receptor on target cells (Figure 4). Since the RBD of S protein directly interacts with the ACE2 receptor on host cells, RBD immunization induced specific antibodies may block this recognition and thus effectively prevent the invasion of the virus. RBD domain is relatively conserved as compared with S1 subunit and was reported to contain multiple conformational neutralizing epitopes, making it more suitable for vaccine development (Jiang S et al., 2005). As a matter of fact, most of SARS-CoV-2 subunit vaccines currently under development use RBD as the antigen. Moreover, the RBD domain was also used in the development of SARS-CoV and MERS-CoV vaccines. For example, studies have demonstrated that recombinant RBD consists of multiple conformational neutralizing epitopes that can induce high titer of neutralizing antibodies against SARS-CoV (Zhu X et al., 2013). Lan et al. reported that Rhesus macaques immunized with the recombinant RBD formulated with alum adjuvant could produce neutralizing antibodies, in association with observed mitigation of the clinical symptoms during MERS-CoV infection (Lan J et al., 2015).

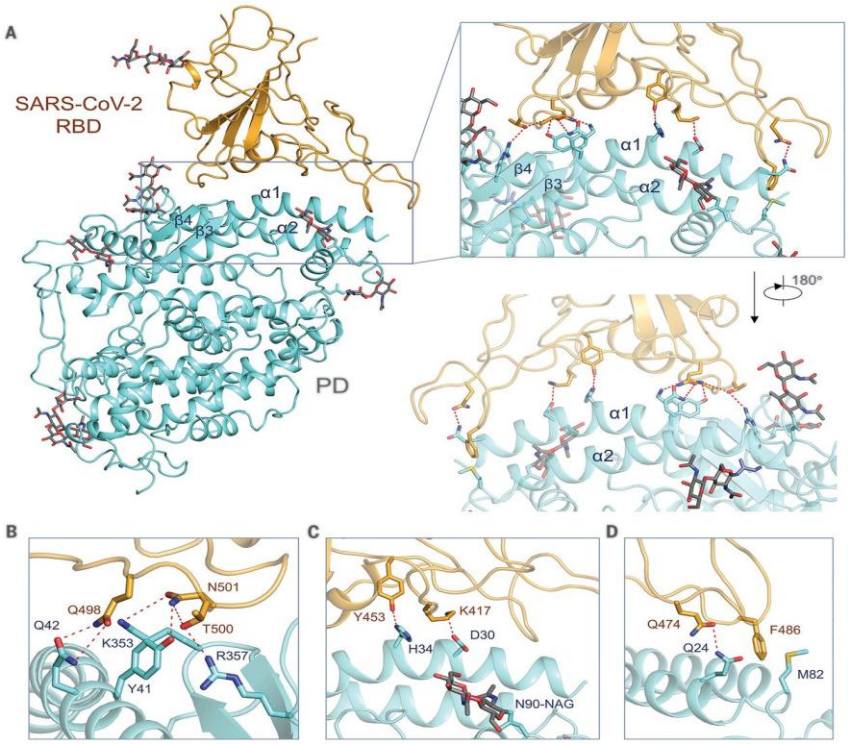


Figure 4: Interactions between SARS-CoV-2-RBD and ACE2. (A) The PD of ACE2 mainly engages the α1 helix in the recognition of the RBD. The α2 helix and the linker between β3 and β4 also contribute to the interaction. Only one RBD-ACE2 is shown. (B to D) Detailed analysis of the interface between SARS-CoV-2-RBD and ACE2. Polar interactions are indicated by red dashed lines. NAG, N-acetylglucosamine.

iii. NTD

Like RBD, the N-terminal domains (NTD) of S protein from several coronaviruses were reported to show carbohydrate receptor-binding activity. One study reported that rNTD of S protein from MERS-CoV induced potent cellular immunity and antigen-specific neutralizing antibodies in mice and was protective against the viral challenge (Jiaming L et al., 2017). However, as the genomes of coronaviruses are highly variable, it is better to use antibodies targeting different epitopes to avoid immune escape of the virus. Although the function of S1-NTD of SARS-CoV-2 has not been elucidated, it may also be involved in the binding of certain receptors and can also serve as a candidate antigen.

iv. S1 subunit

The S1 subunit, which contains both RBD and NTD, is mainly involved in the S protein binding to the host receptor. It is also widely used in vaccine development. Wang et al. reported that MERS-CoV S1 protein formulated with MF59 adjuvant protected hDPP4 transgenic mice against lethal virus challenge, and the protection correlated well with the neutralizing antibody titer (Wang Y et al., 2017).

v. FP

The FP domain of the S2 subunit is involved in the membrane fusion of the virus, which is also a key step in viral pathogenicity. Therefore, it may also serve as a vaccine candidate antigen (Alsaadi EAJ et al., 2019).

3.2 Nucleocapsid protein (N protein)

The N protein is the most abundant protein in coronavirus, and it is normally highly conserved, with a molecular weight of about 50 kDa. N protein has multiple functions including formation of nucleocapsids, signal transduction virus budding, RNA replication, and mRNA transcription (McBride R et al., 2014). This protein was reported to be highly antigenic, 89% of patients who developed SARS, produced antibodies to this antigen (Leung DT et al., 2004). N protein-based vaccines usually cannot induce neutralizing antibodies, likely due to the fact that N protein is not displayed on the CoV surface. However, N protein has the advantage of being more conserved across CoV species than S protein, making it a potential target for a T-cell inducing, universal CoV vaccine (Wang N et al., 2020). One recent study has shown that a viral vector vaccine expressing N protein can induce $CD4^+$ T cell-dependent protection against SARS-CoV and MERSCoV, suggesting the feasibility of N protein-based T-cell inducing CoV vaccines (Zhao J et al., 2016).

3.3 Membrane protein (M protein)

The M protein of coronavirus plays a central role in virus assembly, turning cellular membranes into workshops where virus and host factors come together to make new virus particles. It is a trans-membrane glycoprotein with a molecular weight of about 25 kDa (Neuman BW et al., 2011). It was reported that immunization with the full length of M protein is able to elicit efficient neutralizing antibodies in SARS patients. Immunogenic and structural analysis also indicated that the trans-membrane domain of the M protein contains a T cell epitope cluster that is able to induce a strong cellular immune response (Liu J et al., 2010). M protein is also highly conserved in evolution among different species; therefore, it may be used as a candidate antigen for developing SARS-CoV-2 vaccine.

3.4 Envelope protein (E protein)

E gene encodes a small multifunctional protein that possesses ion channel (IC) activity, an important function in virus-host interaction. SARS-CoV E protein is a 76-amino acid transmembrane protein actively synthesized during viral infection, that mainly localizes at the ERGIC region of the cell, where virus budding and morphogenesis take place. Different requirements of E protein during the virus cycle have been described among CoVs (Pervushin K et al., 2009). Compared with S, N, and M protein, E protein is not suitable for use as an immunogen. Studies have shown that SARS-CoV E protein is an important virulence factor, and the secretion of inflammatory factors IL-1, TNF, and IL-6 are significantly reduced after knocking out E protein (Nieto-Torres JL et al., 2014).

4. VACCINE DEVELOPMENT STRATEGIES AND PLATFORMS

Viral zoonosis had come about into numerous disease epidemics in recent years and development of new strains from their zoonotic hosts makes them exceptionally hard to foresee (Lau SK et al., 2005). Prior, CoV were thought as powerless pathogen for people causing mild influenza like sickness but with consistent episodes like SARS in 2002, MERS in 2012 and now COVID-19 their pathogenicity is very grounded internationally (Chan JF et al., 2015). Such repeated transmission prompting worldwide economy misfortunes makes CoV vaccines profoundly desirable, as of now there are no antiviral medications avilable against CoV. Much effort is being made to develop vaccine against SARS-CoV2 on accounts to tackle the current coronavirus pandemic. Different regions investigated for the inquiry of an ideal immunization against SARS-CoV, includes inactivated virus vaccines, recombinant viral vaccines, protein subunit vaccine, DNA vaccines, RNA vaccines, non-replicating viral vector, replicating viral vector, and live-attenuated vaccines (Figure 5) (Kyriakidis NC et al., 2021). Each approach has advantages and disadvantages (Table 1) (Li YD et al., 2020). As the SARS-CoV-2 shares similarities in the genetic makeup of two deadly coronaviruses, i.e. SARS and MERS, vaccine strategies used to combat SARS and MERS viruses are being adopted to guide the formulation and development of new SARS-CoV-2 vaccines (Giovanni Salvatori et al., 2020).

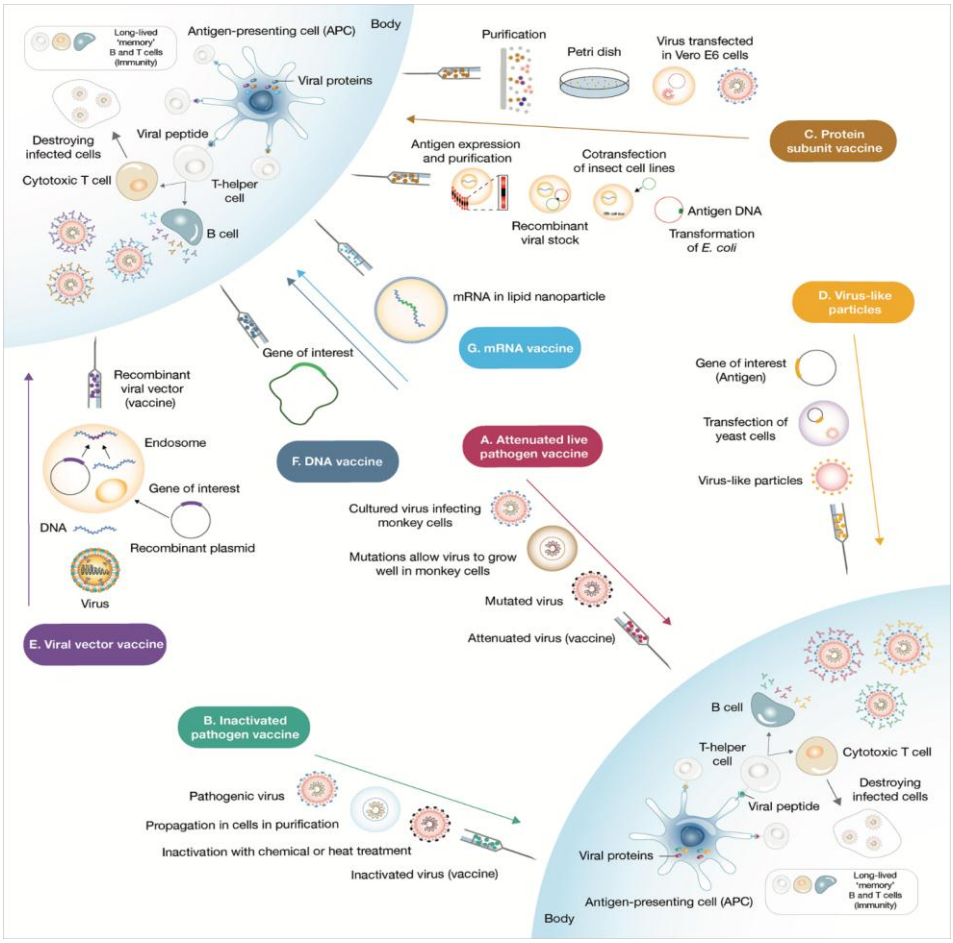


Figure 5: Overview of the strategies used for vaccine development and delivery.

Table 1: Advantages and disadvantages of different vaccine platforms (Li YD et al., 2020).

Vaccine platform	Advantages	Disadvantages
Whole inactivated virus vaccine	Stronger immune response; Safer than live attenuated virus	Potential epitope alteration by inactivation process
Live attenuated virus vaccine	Stronger immune response; Preservation of native antigen; Mimicking natural infection	Risk of residual virulence, especially for immunocompromised people
Viral vector vaccine	Stronger immune response; Preservation of native antigen; Mimicking natural infection	More complicated manufacturing process; Risk of genomic integration; Response dampened by pre-existing immunity against vector
Subunit vaccine	Safe and well-tolerated	Lower immunogenicity; Requirement of adjuvant or conjugate to increase immunogenicity
Viral-like particle vaccine	Safe and well-tolerated; mimicking native virus conformation	Lower immunogenicity; More complicated manufacturing process
DNA vaccine	Safe and well-tolerated; Stable under room temperature; Highly adaptable to new pathogen; Native antigen expression	Lower immunogenicity; Difficult administration route; Risk of genomic integration
RNA vaccine	Safe and well-tolerated; Highly adaptable to new pathogen; Native antigen expression	Lower immunogenicity; Requirement of low temperature storage and transportation; Potential risk of RNA-induced interferon response

4.1 Live-attenuated vaccines

Live attenuated vaccines are produced by generating a genetically weakened version of the virus that replicates to a limited extent, causing no disease but inducing immune responses that are similar to that induced by natural infection (Figure 6). This type of vaccines usually elicits robust and long-term memory immune responses after a single dose. Attenuation can be achieved by adapting the virus to unfavourable conditions (for example, growth at lower temperature, growth in non-human cells) or by rational modification of the virus (for example, by codon de-optimization or by deleting genes that are responsible for counteracting innate immune recognition) (Broadbent AJ et al., 2016). An important advantage of these vaccines is that they can be given intranasally, after which they induce mucosal immune responses that can protect the upper respiratory tract—the major entry portal of the virus. In addition, because the virus is replicating in the vaccinated individual, the immune response is likely to target both structural and non-structural viral proteins by way of antibodies and cellular immune responses. However, disadvantages to these vaccines include safety concerns and the need to modify the virus, which is time-consuming if carried out by traditional methods and technically challenging when reverse genetics is used. Deletion of the structural E gene may prove to be the first step in the field of developing a live-attenuated vaccine against SARS‑CoV or MERS-CoV (DeDiego ML et al., 2007). Two attenuated SARS-CoV-2 virus vaccine candidates are in pre-clinical development currently – the first a joint effort by US company Codagenix and the Serum Institute of India, and the second run by Indian Immunologicals Ltd and Australia's Griffith University. They use viral deoptimization to synthesize "rationally designed" live-attenuated vaccines (WHO, 2020).

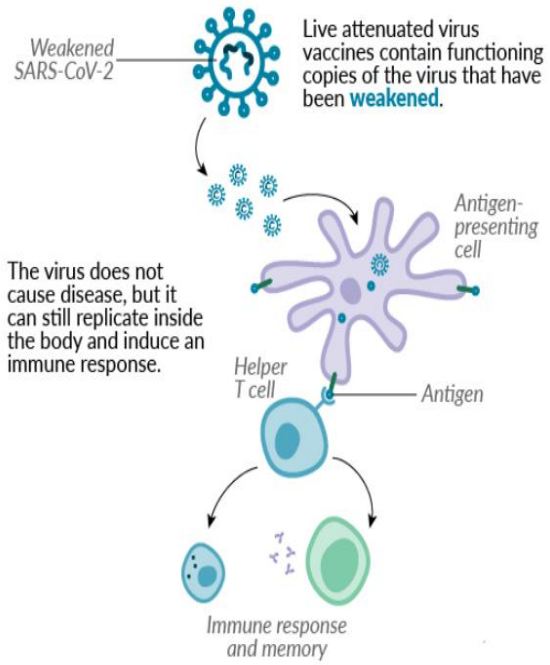


Figure 6: The way live attenuated virus vaccines act.

4.2 Inactivated virus vaccines

Inactivated virus vaccines also known as the WKV (whole Killed Virus) vaccines represent a pathogen whose ability to infect and replicate has been ceased, consequently making it sterile but retaining its ability to act as an immunogen, so that the immune system could still work if such a pathogen is injected into a host (Figure 7). Inactivated vaccines are prepared by neutralizing the pathogen as a whole by chemicals or by heat and radiation. It is thought that inactivated vaccines can be prepared with much less effort which makes them one of the attractive types of vaccines prepared in the market today. These vaccines work by exposing the same epitopes which a virus otherwise would have presented, thus eliciting an immune response. Inactivated SARS-CoV has been tested in humans and found to be safe and elicited SARS-CoV specific neutralizing antibodies, however the efficiency the vaccine in humans yet to be reported (Lin JT et al., 2007). These data suggested that inactivated vaccines are safe and they are able to induce SARS-CoV specific neutralizing antibodies so inactivated viral vaccines could also be evaluated as potential vaccine candidate against SARS-CoV-2. Several inactivated vaccine candidates have entered clinical trials, with three candidates from China in phase III trials, and one from India, one from Kazakhstan and two from China in phase I or II clinical trials (WHO, 2020).

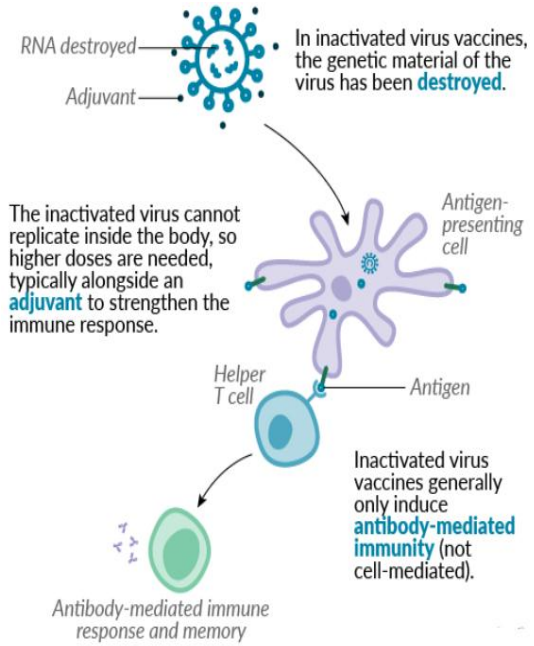


Figure 7: The way inactivated (killed) virus vaccines act.

4.3 Viral-vector based vaccines

Viral vector vaccines are recombinant viruses that encode antigens of interest in an unrelated modified virus. They deliver antigen into the cells mimicking natural infection, so they induce strong antigen-specific cellular and humoral immune responses per se, thereby obviating the need for additional adjuvants. In addition,

viral vectors are able to accept large insertions in their genome, providing a flexible platform for antigen design. Despite these advantages, there are several drawbacks. The manufacturing process for viral vector vaccines is more complicated than other approaches, including the optimization of cellular systems and the exclusion of contaminants, which can greatly affect the efficiency of viral vectors (Rauch S et al., 2018). Moreover, recombinant viruses carry the risk of integrating their genome into the human host, so additional biosafety assessment will be required before entering clinical trials. Finally, if the chosen viral vector can infect the general populations, the pre-existing immunity on the viral vector could dampen the induced immune response, which has been seen in adenovirus and measle virus-based vaccines (Fausther-Bovendo H et al., 2014).

There are two broad viral vector groups used for vaccine production, namely replication-competent and replication-incompetent viral vectored vaccines (Figure 8). Replication-competent vectors need lower dose to elicit strong responses as the multiplying vector can result in enhanced antigen presentation. Conversely, replication- incompetent vectors should be administered in higher dosages since they are devoid of a self-propagation capacity. However, this last characteristic allows translating into safer platforms.

Most viral vector coronavirus vaccines target the S antigen. Several attempts have been made in the direction of the development of SARS coronavirus (SARS-CoV) vaccine for which various viral vectors are genetically engineered to express SARS-CoV proteins in them (Table 2). Adenovirus and modified vaccinia virus Ankara (MVA) are the two most common viral vectors used in the development of SARS-CoV and MERS-CoV vaccines. Related SARS-CoV-2 vaccine research has been carried out by the following institutions. Houston-based Greffex Inc. has completed the construction of SARS-CoV-2 adenovirus vector vaccine with Greffex Vector Platform and should have now moved to animal testing. Tonix Pharmaceuticals announced research to develop a potential SARS-CoV-2 vaccine based on Horsepox Virus (TNX-1800). Johnson & Johnson has adopted the AdVac® adenoviral vector platform for vaccine development (Gonzalez-Nicolini V et al., 2006).

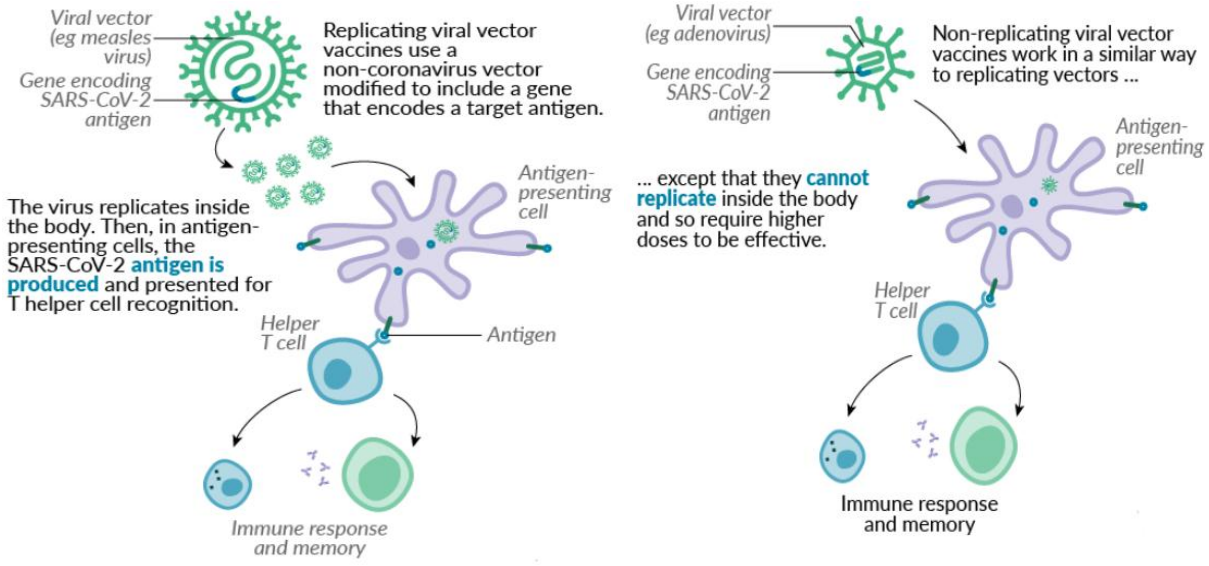


Figure 8: The way replication-competent and replication-incompetent viral vector vaccines act.

Table 2: Replication-competent and replication-incompetent viral vectors investigated as vaccine candidates against SARS-CoV-2 (Malik JA et al., 2021).

Replication-competent vectors	Replication-incompetent vectors
YF17D Vector Measles Vector Horsepox vector LVVV based on attenuated influenza virus backbone Influenza vector Replication-competent VSV chimeric virus technology Newcastle disease virus vector Avian paramyxovirus vector	Sendai virus vector Adenovirus-based MVA encoded VLP Replication defective Simian Adenovirus adenovirus-based NasoVAX adenovirus-based + HLA-matched peptides Inactivated Flu-based SARS-CoV2 vaccine + Adjuvant Influenza A H1N1 vector parainfluenza virus 5 -based vaccine Recombinant deactivated rabies virus Dendritic cell-based vaccine

4.4 Subunit vaccines

The principle underlying the development of subunit vaccines was based upon the observation that do not need to administer the entire pathogen to elicit strong immune responses, but merely an immunogenic fragment (Figure 9). Subunit vaccines are prepared either from antigen purification of pathogens replicated in cell cultures or from recombinantly expressed antigens. These vaccines commonly require adjuvant addition in order to deliver danger signals to antigen-presenting cells and provoke robust immune responses. Since there is no live fragment involve, there is no danger of prompting a disease (B. Sarkar et al., 2019). Subunit antibodies can be additionally classified into protein-based subunit vaccines, polysaccharide vaccines, and conjugate subunit vaccines. Protein subunit vaccines are more steady and safer than live attenuated and inactivated/killed vaccines. They can be manufactured in a more cost-efficient manner as compared to other types of vaccines. A shortcoming of this strategy is that if isolated proteins get denatured, may bind to different antibodies than the targeted protein of the pathogen (Wang M et al., 2016).

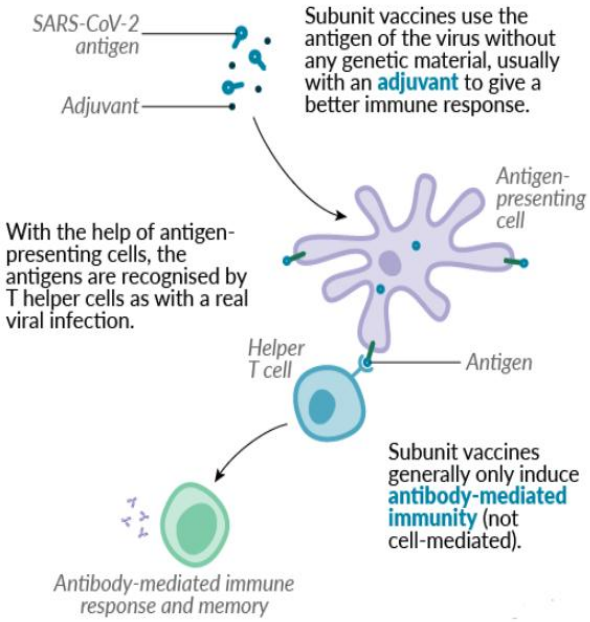


Figure 9: The way subunit vaccines act.

Protein subunits comprising spike (S), envelope (E), membrane (M), and nucleocapsid (N) that are expressed by SARS-CoV-2 are viral antigens actuate neutralizing antibodies and generate a protective immune response (Pandey SC et al., 2020). To develop vaccines based on CoV subunit, S proteins are the preferred candidate, because S proteins have sites for binding of receptor as well as for membrane fusion; vaccines based on S protein are likely to activate antibodies that prevent the binding of virus and later fusion of membranes as well thereby counteracting infection of virus. S-ectodomain proteins of full length merged with/ without S or S fold on i.e. fold on trimeric motif, may induce precise antibody response and neutralizing antibody thus causing protection of the vaccinated mice to SARS-CoV infection (Li J et al., 2013). Besides this, S protein associated with SARS-CoV is also shown to be responsible for eliciting responses by T-cell ($CD4^+$ and $CD8^+$) (Huang J et al., 2007).

RBD is a segment in the centre of S1 subunit of S protein which is ~193 aa residues and binds to receptors present in the target cells. It has been illustrated that antisera of SARS infected person and that of animals inoculated with inactivated SARS-CoV, effectively responded with RBD (He Y et al., 2004a). As compared to S protein of full length, high number of neutralizing antibodies were induced with RBD because in contrast to S protein with full length, no immunodominant regions are present in RBD that elicits antibodies that are not neutralizing (He Y et al., 2004b). Thus, the generation of antibodies targeting the RBD subunit of SARS-CoV-2 would be an important preventive and treatment strategy that can be tested further in suitable models before clinical trials.

So far, several institutions have initiated programs on the SARS-CoV-2 subunit vaccine. For example, Intravacc.in collaboration with EpiVax are working on the outer membrane vesicle (OMV) delivery platform with synthetically produced SARS-CoV2 epitopes. It is one of the diverse platforms that are being investigated to produce a subunit vaccine. The candidate is currently under pre-clinical evaluation. Other diverse novel platforms that are being investigated under this strategy include GP-96 backbone and li-key peptide. Besides, Johnson & Johnson, Pasteur Institute, and Chongqing Zhifei Biological Products Co., Ltd. also started subunit vaccine development against SARS-CoV-2 (WHO, 2020).

4.5 Virus-like-particle vaccines

Virus-like particles (VLPs) are self-assembled viral structural proteins that mimic the conformation of native viruses but lack the viral genome. Compared with protein subunit vaccines, VLP vaccines present epitope in conformation that is more similar to the native virus, leading to better immunization responses (Figure 10). In addition, compared to whole virus vaccines, the production of VLP vaccines does not involve live virus or inactivation steps, which makes them safer vaccine candidates. The highly repetitive antigenic surface of VLP vaccines also help induce stronger antibody response by efficiently cross-linking B-cell surface receptors (Hill BD et al., 2018). VLP's are the newest vaccine development platform. They are further classified as enveloped and non-enveloped VLP's based on their structure. Enveloped VLP's consist of the cell membrane of the host cell called an envelope and this envelope contains integrated target antigens displayed on the surface (B. Sarkar et al., 2019).

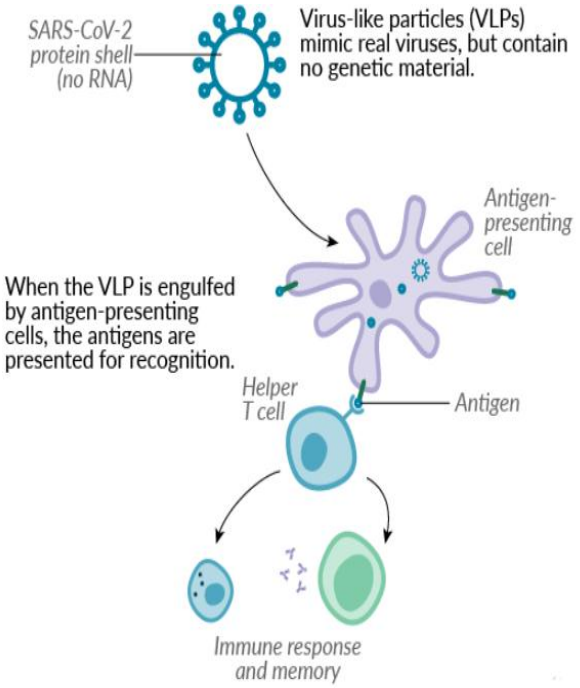


Figure 10: The way virus-like-particle vaccines act.

Few SARS-CoV and MERS-CoV VLP vaccines have been reported so far. For SARS-CoV, Lokugamage et al. have demonstrated that chimeric VLPs composed of SARS-CoV S protein and mouse hepatitis virus E, M and N proteins can induce neutralizing antibody responses and reduce SARS-CoV virus titer in mice lung after viral challenge (Lokugamage KG et al., 2008). For MERS-CoV VLP vaccines, Wang et al. have shown that VLPs containing MERS-CoV S, E and M proteins can induce specific antibody response and Th1-mediated cellular immunity in rhesus macaques (Wang C et al., 2017a). The same research group developed another chimeric VLP vaccine containing the fusion of the receptor-binding domain (RBD) of MERS CoV S protein and the canine parvovirus (CPV) VP2 structural protein. They showed that this VLP vaccine induces MERS-CoV-specific antibody response and T-cell immunity in mice (Wang C et al., 2017b). These studies suggested that VLP vaccines hold the potential for clinically effective coronavirus vaccines.

Up to now, there have been 13 SARS-CoV-2 protein subunit vaccines entering clinical trials (WHO, 2020). Among these vaccines, a leading company Novavax, with its NVX-CoV2373 vaccine, has entered a phase IIb trial in South Africa (NCT04533399) and a phase III trial in the UK (2020-004123-16). NVX-CoV2373 contains a prefusion stabilized full-length spike protein adjuvanted with their proprietary saponin-based adjuvant (Novavax, 2020).

4.6 Nucleic acid vaccines

Nucleic acid (DNA and mRNA) vaccines are very quick to produce, yet were untested as successful human vaccine strategies. Antigen that encodes either plasmid DNA or RNA i.e. mRNA or viral replicon, are used in the nucleic acid based approaches. After being taken and expressed by a cell, these antigens, which are encoded by nucleic acid, induce antibody and cell -mediated response as well. Owing to the simplicity in the alteration of antigen they permit, both the approaches are tremendously adaptable. Antigen production in the target cells suggests the benefit of imitating synthesis of protein throughout infectivity. Prominently, they allow any preference antigen delivery, despite of the fact that it was either isolated from bacteria, virus or any parasite thereby permitting development of vaccine against broad pathogen group. Again vaccine characteristics are not dependent on encoded proteins so, there is no need to set up new production, purification, validation methods and manufacturing services for production of nucleic acid-based vaccines in large scale. Furthermore, these vaccines are predicted to have minor safety issues as nucleic acid is swiftly degraded within the human body.

4.6.a DNA vaccines

DNA vaccines contain genes encoding viral antigenic components that are expressed by plasmid vectors and delivered into cells through electroporation. Compared with other vaccine technologies, DNA vaccines offer a fast and flexible platform for vaccine development and production, making it an attractive technology to combat emerging epidemics like SARS-CoV-2. In addition, antigen production of DNA vaccines happens in the target cells, which helps recapitulate the native conformation and post-translational modification of viral antigens (Figure 11). DNA molecules are generally quite stable, permitting the storage of DNA vaccines at +4 °C, thereby simplifying the distribution of this type of vaccines. However, an important drawback of DNA vaccines is their limited immunogenicity due to their inability to spread and amplify in vivo. Therefore, it is important to consider strategies that can enhance the potency of DNA vaccines, such as adding adjuvant or using a prime-boost regimen. Besides, the genomic integration of DNA vaccines into the host chromosome is another biosafety concern, which may lead to mutagenesis and oncogenesis (Rauch S et al., 2018).

Several DNA vaccine candidates have been reported for SARS-CoV, including the S-, M-, and N protein-based vaccines (Wang Z et al., 2005). Although all of them can generate a certain level of antibody and cell-immune responses, only S protein-based DNA vaccine has been shown to induce protective effect against SARS-CoV infection, probably due to the indispensable role of S protein in receptor binding. Yang et al. has demonstrated that immunization with DNA encoding full-length S protein, S protein lacking part of cytoplasmic domain, S protein lacking both cytoplasmic and transmembrane domains can all induce neutralizing antibodies and T-cell immune responses, as well as providing protective effect in mice (Yang ZY et al., 2004). This promising result leads to a following phase I clinical trial based on SARS-CoV full-length S protein DNA vaccine, which showed that the vaccine was well-tolerated in patients and can

induce neutralizing antibodies and T cell responses in healthy adults (Martin JE et al., 2004).

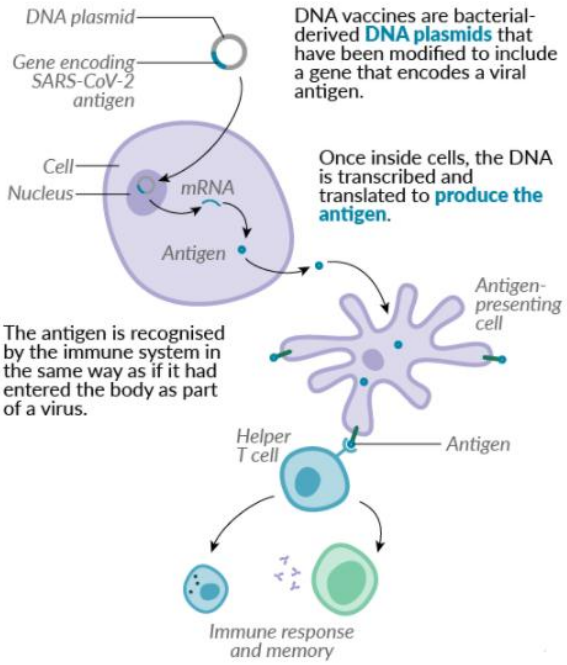


Figure 11: The way DNA vaccines act.

Similar to SARS-CoV, several studies on MERS-CoV DNA vaccines have demonstrated optimistic results. Muthumani et al. reported that a full-length S protein-based MERS-CoV DNA vaccine can induce potent cellular immunity and antigen-specific neutralizing antibodies in mice, macaques, and camels, and macaques vaccinated with this DNA vaccine were protected against MERS-CoV challenge without demonstrating any clinical or radiographic signs of pneumonia (Muthumani K et al., 2015). Building on these encouraging data, a phase I clinical trial based on this MERS-CoV DNA vaccine (GLS-5300, or INO-4700) has been completed. The results showed that GLS-5300 is well tolerated with no vaccine-associated serious adverse events, and immunization with GLS-5300 induces durable immune responses in 85% of participants after two vaccinations (Modjarrad K et al., 2019). These data support further development of the GLS-5300 vaccine. Notably, a SARS-CoV-2 DNA vaccine candidate, INO-4800, is based on the same design as GLS-5300, and this vaccine is now in phase I/II clinical trial (NCT04447781 and NCT04336410) (Smith TRF et al., 2020).

Taken together, DNA vaccines encoding full-length S or S1 protein have demonstrated encouraging results to fight against SARS-CoV and MERS-CoV. The same strategy is likely to be generalizable to SARS-CoV-2 DNA vaccine considering the biological similarity. So far, two SARS-CoV-2 DNA vaccines are under development. Inovio Pharmaceuticals developed a DNA vaccine candidate termed INO-4800, which is in preclinical studies and will soon enter phase I clinical trials. Applied DNA Sciences Subsidiary, LineaRx, and Takis Biotech collaborated for the development of a linear DNA vaccine candidate against SARS-CoV-2, which is now in preclinical studies (WHO, 2020).

4.6.b RNA vaccines

RNA vaccines consist of viral antigen-encoding messenger RNAs that can be translated by human cells to produce antigenic proteins and stimulate the immune system. RNA vaccines are usually delivered in complex with additional agents, such as protamine or lipid- and polymer-based nanoparticles, to increase its efficacy (Kauffman KJ et al., 2016). Similar to DNA vaccines, RNA vaccines have the advantages of being highly adaptable to new pathogens and being able to recapitulate the native conformation and modifications of antigenic proteins (Figure 12). Furthermore, compared with DNA vaccines, RNA vaccines have some additional benefits. Unlike DNA, RNA does not interact with host-cell DNA and therefore obviate the risks of genomic integration. Besides, RNA vaccines can be given through multiple routes including traditional intravenous injection, whereas DNA vaccines need to be administered via special devices like electroporation or gene gun. Nevertheless, RNA vaccines do have some drawbacks. Exogenous RNA can activate interferon-mediated antiviral immune response and lead to stalled translation and mRNA degradation, which suppress the efficacy of RNA vaccines (Sahin U et al., 2004). In addition, interferon signaling is associated with inflammation and potential autoimmunity (Pardi N et al., 2018). Even though there have not been severe cases of RNA vaccine-induced autoimmune diseases, it is important to carefully evaluate this potential adverse effect.

Although there were no RNA vaccine studies for SARS-CoV or MERS-CoV in the past two decades, there have already been 6 novel RNA vaccines reaching clinical trials for SARS-CoV-2 since the outbreak of COVID-19. So far, a SARS-CoV-2 mRNA vaccine (mRNA-1273, encoding S protein) developed by Moderna, has been launched in animal experiments and clinical batch production. It is expected that clinical trials will be conducted on 20–25 healthy volunteers by the end of April. Fudan University is in collaboration with Shanghai Jiaotong University and Bluebird Biopharmaceutical Company to develop a SARS-CoV-2 mRNA vaccine using two different strategies. The first is to use mRNA to express the SARS-CoV-2 S protein and RBD domain, the efficacy of this vaccine is now under evaluation in mice. The second is the use of mRNA to express virus-like particles in vivo. In addition, German biopharmaceutical company CureVac AG, Stermirna Therapeutics, BDGENE Therapeutics, Guanhao Biotech, ZY Therapeutics Inc., CanSino Biologics Inc., Baylor College of Medicine, University of Texas, Tongji university also announced their progress on mRNA vaccine development against SARS-CoV-2 (WHO, 2020).

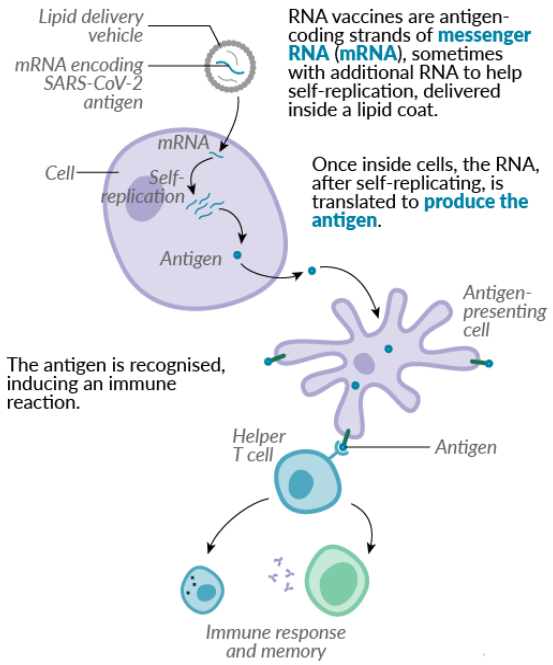


Figure 12: The way RNA vaccines act.

4.7 Adjuvant

Adjuvants incorporated in prophylactic and/or therapeutic vaccine formulations impact vaccine efficacy by enhancing, modulating, and/or prolonging the immune response (Figure 13). In addition, they reduce antigen concentration and the number of immunizations required for protective efficacy, therefore contributing to making vaccines more cost effective. Adjuvants are critical components of both subunit and certain inactivated vaccines because they induce specific immune responses that are more robust and long-lasting. In addition, for live attenuated vaccines and live vector vaccines, adjuvants are required to enhance the immune response in the development of vaccines (Bonam SR et al., 2017). A review of the history of coronavirus vaccine development demonstrates that only a few adjuvants, including aluminum salts, emulsions, and TLR agonists, have been formulated for SARS-CoV and MERS-CoV vaccines in experimental and pre-clinical studies.

Vaccine development utilizing various platforms is one of the strategies that has been proposed to address COVID-19 pandemic. In order to accelerate the development of a SARS-CoV-2 vaccine, the preferred adjuvant should be those have been widely used in other marketable vaccines, including classic aluminum adjuvant, aluminum adjuvants enhance the immune response by facilitating phagocytosis and slowing the diffusion of antigens from the injection site. It can efficiently stimulate Th2 immune response upon injection (Hogenesch H, 2013). In addition, MF59, MF59 is an oil-in-water emulsion composed of Tween 80, sorbitol trioleate, and squalene, and it has already been used in flu vaccines in Europe and the United States. The mechanism of MF59 is to create a transient immune environment at the injection site, then to recruit immune cells to induce antigen-specific immune responses (Tsai TF, 2013). Currently, GSK announced that they would make its established pandemic vaccine adjuvant platform technology available to enhance the development of an effective vaccine against SARS-CoV-2, and agreements have been reached with Clover Biopharmaceutical Inc. and the University of Queensland, Australia. Because adjuvants were able to regulate the type of immune response, the optimal adjuvant should be selected according to the design of the vaccine. In order to induce a more efficient immune response, a combination of different types of adjuvants could be applied to improve the immune efficacy.

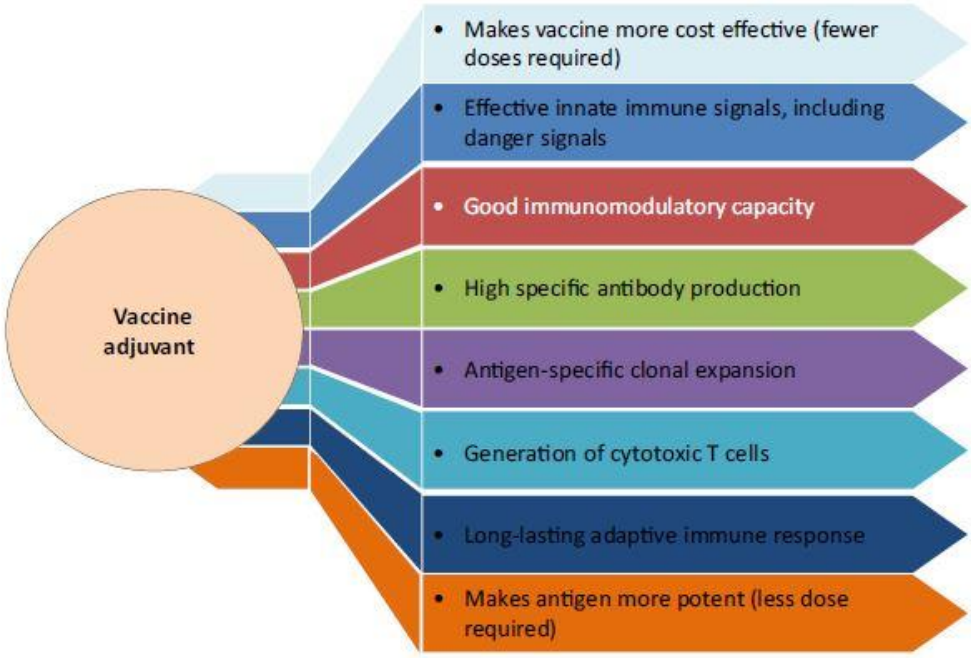


Figure 13: Characteristic Properties of Vaccine Adjuvants.

5. MECHANISM OF ACTION OF VARIOUS VACCINE CANDIDATES

The most effective licensed vaccines elicit long-term antigen-specific antibody responses by plasma cells in addition to the development of persisting T cell and B cell memory. In case of SARS-CoV infection both humoral and cellular immune responses are crucial for the clearance of infection (Figure 14). Recombinant virus vectors work in a similar manner like an endogenous pathogen, by expressing axenic target protein in cytoplasm of the host cell. After, processing of such endogenous antigen, MHC class 1 molecules present them to $CD8^+$ T-lymphocytes, which causes production of T-cytotoxic cells. This pathway, leads to establishment of cell-mediated immunity, which is crucial in getting rid of virus infected cells. Sub unit vaccine candidate particularly RBD of S protein of SARS-CoV contains major antigenic determinants that can induce neutralizing antibodies (Bonavia A et al., 2003). The SARS-CoV S protein can also induce $CD8^+$ T-cell responses. The RBD of S protein contains multiple conformation-dependent epitopes and is the main domain that induces neutralizing antibody and T-cell immune responses against SARS-CoV infection making it an important target for vaccine development (He Y et al., 2006). The approaches for developing RBD-based vaccines against SARS-CoV have provided useful information for designing safe and effective vaccines against SARS-CoV-2 since RBDs of SARS-CoV-2 also contain similar epitopes. Similarly, Adenoviral vectors are able to induce potent antibody as well as T cell responses with variations in the immune response depending on the serotype employed. Replication deficient Ad5, one of the most widely used adenoviral vectors, is able to induce exceptionally potent $CD8^+$ T cell as well as antibody responses (Humphreys IR et al., 2018).

Furthermore, DNA vaccination is also able to elicit both humoral and cellular immune responses, through activation of $CD8^+$ cytotoxic and $CD4^+$ helper T cells, respectively. Upon entry in the cell, DNA vaccines are sensed by a variety of innate immune receptors i.e. STING/TBK1/IRF3 pathways and the AIM2 inflammasome and many other factors are involved in DNA vaccine mode of action but the exact mechanism of action is yet to be evaluated. However, immunization with S protein encoding DNA vaccine elicited protective immunity against SARS-CoV infection in a mouse model by inducing T cell and neutralizing antibody responses (Yang ZY et al., 2004). Another nucleotide-based vaccine i.e. Exogenous mRNA is also immunostimulatory, as it is recognized by a variety of cell surface, endosomal and cytosolic innate immune receptors. Mammalian cells can sense foreign RNA via Pattern recognition receptors (PRRs) such as TLR3, TLR7 and TLR8 located in the endosomes and RIG-I, MDA-5 and PKR located in the cytoplasm as well as NLRP3 and NOD2 (Chen N et al., 2017). Activation of the PRRs by mRNA vaccines results in a robust innate immune response including production of chemokines and cytokines such as IL-12 and TNF at the inoculation site, which are innate factors crucial for the induction of an effective adaptive immune response against the encoded antigen. The mRNA vaccines can also induce an immunological repertoire associated with the generation of high magnitude long-lived antibodies (Edwards DK et al., 2017).

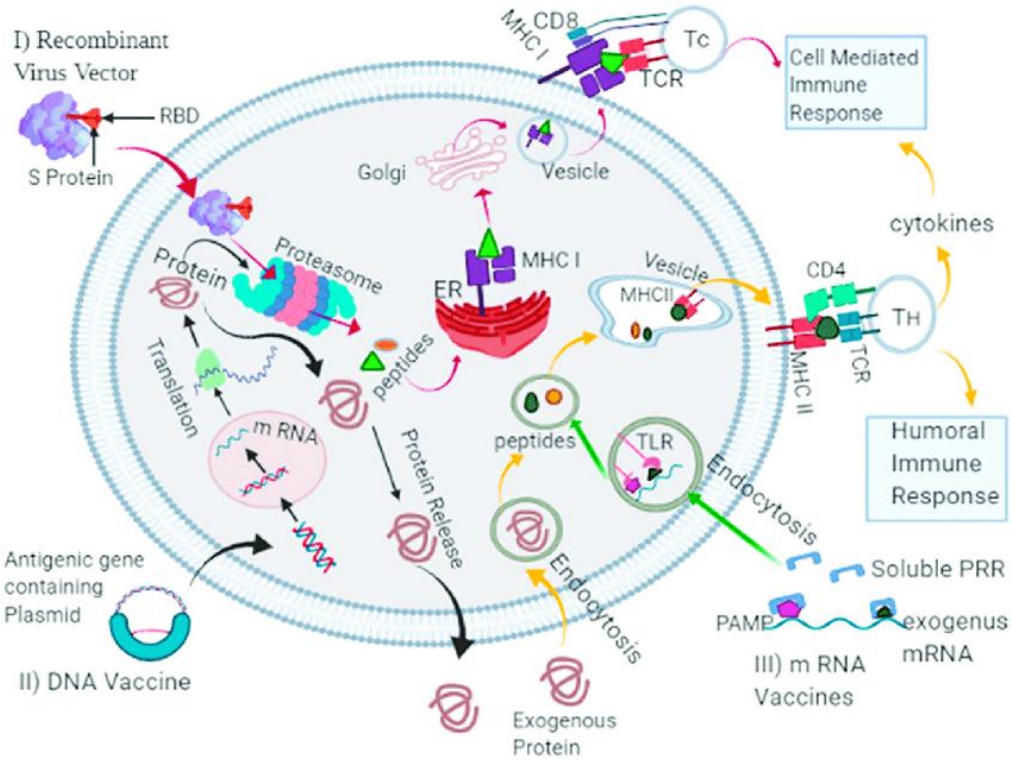


Figure 14: Mechanism of action of various vaccine candidates.

6. PREVIOUS PROGRESS OF SARS-COV AND MERS-COV IMMUNIZATION STRATEGIES

6.1 Vaccines for SARS-CoV

After the SARS epidemic in 2002–2003, several laboratories around the world started to conduct vaccine development studies for preventing the disease (Table 3). The majority of the subunit vaccines targeted the spike (S) glycoprotein of the virus. SARS-CoV uses this glycoprotein to bind and enter the host cells (Du L et al., 2009). Therefore, a vaccine that induces strong immune responses against this protein will have a significant effect on the deterrence of virus entry to the host cells during natural infection.

Vaccines based on a live-attenuated or inactivated virus, recombinant viral vectors, DNA, virus-like particles (VLPs) and soluble proteins were studied, mainly in pre-clinical studies. Live-attenuated and inactivated viruses are based on the use of the whole SARS-CoV as a vaccine. The virus has been rendered nonreplicating, and infectivity has been greatly reduced by means of deleting components of the virus genome or by using physical or chemical methods (Petrovsky N et al., 2004). In the case of recombinant viral vectors, viruses different from the SARS-CoV that are capable of host cell infection have been genetically engineered to express components of the SARS-CoV (Lauer KB et al., 2017). VLPs are non-infectious multiprotein structures formed from viral proteins that self-assemble into virus-like structures (Urakami A et al., 2017). So far, only vaccines based on an inactivated SARS virus, DNA and soluble proteins based on the SARS S glycoprotein reached a clinical stage (phase I) (Lin JT et al., 2007; Martin JE et al., 2008; NIH, 2013).

6.2 Vaccines for MERS-CoV

Several vaccines have been developed for MERS coronavirus since its emergence in 2012 (Table 3). As in the case of the SARS vaccines, most of the subunit vaccines for MERS are based on the S glycoprotein. Vaccines based on inactivated and live attenuated viruses, recombinant viral vectors, nanoparticles, DNA and soluble proteins have been developed and tested predominantly in animal models. Up to date, only a DNA-based vaccine has already been tested in clinical trials (phase I) (Modjarrad K et al., 2019) with other vaccines such as MVA (modified vaccinia virus Ankara) and adenoviruses being currently under study at that clinical stage (NIH, 2019).

Table 3: Different types of vaccination strategies against SARS-CoV and MERS-CoV (Pandey SC et al., 2020).

Vaccines strategies	Vaccine candidates	Phase
Nucleotide based	DNA vaccines S, M and N genes	Phase I, II (NCT03721718)
	mRNA vaccines mRNA -1273 and BNT162 encoding S protein	Phase I (mRNA-1273) and Preclinical (BNT162)
Subunit vaccine	Spike glycoproteins (S), Membrane proteins (M), Nucleoproteins (N)	Preclinical
Recombinant Vector Vaccines	Coronavirus proteins/glycoproteins expressed by attenuated adenovirus/poxvirus/newcastle disease virus	Phase I (NCT03399578, NCT03615911
Attenuated vaccines	Gene deletion of various essential genes (S,N,E genes), Nonstructural proteins (nsp) encoding genes	Preclinical
Inactivated virus vaccines	Inactivated or whole killed virus (WKV)	Preclinical

7. RECENT PROGRESS ON SARS-COV-2 VACCINE DEVELOPMENT

Since the publication of the genome sequence of SARS-CoV-2, on January 11th, 2020, an endeavor of unprecedented speed and magnitude set out to develop a vaccine against the disease (Table 4). Early scientific opinions predicted that it would take at least a year to a year and a half to get a SARS-CoV-2 vaccine approved for use in the United States. Still, recent advances on the field have made possible the issuing of emergency use authorizations (EUAs) by several national and international drug regulation agencies for different vaccine candidates against SARS-CoV-2 in less than a year since the virus genome sequence was released. An ideal SARS-CoV-2 vaccine should meet the following requirements: protect not only from severe disease but also thwart infection in all vaccinated populations, including less immunocompromised individuals, elicit long term memory immune responses after a minimal number of immunizations or booster doses, the manufacturing company should be able to ramp up production to produce billions of doses annually and have the potential to make it easily accessible for worldwide vaccination campaigns at an affordable cost and at limited time (AEP, 2020).

Table 4: COVID-19 vaccine in phase I and II clinical trials (Poland GA et al., 2020).

Vaccine Developers	Vaccine type	Location	Trial number
Phase 1 trials only			
Inovio	DNA (INO-4800)	USA	NCT04336410
Genexine	DNA (GX-19)	South Korea	NCT04445389
Academy of Military Sciences; Suzhou Abogen Biosciences; Walvax Biotechnology	mRNA (ARCoV)	China	··
ReiThera; Lazzaro Spallanzani National Institute for Infectious Diseases	Gorilla adenovirus vector (GRAd-CoV2)	Italy	NCT04
Clover Pharmaceuticals; Dynavax Technologies	Protein (SCB-2019)	··	NCT04405908
Vaxine	Protein	Australia	NCT04453852
Medicago; GSK; Dynavax Technologies	Virus-like particle	USA	NCT04450004
University of Queensland; CSL	Proteins	Australia	NCT04495933
Kentucky Bioprocessing	Plant	USA	NCT04473690
Medigen; Dynavax Technologies	Protein (MVC-COV1901)	Taiwan	NCT04487210
Adimmune	Protein (AdimrSC-2f)	Taiwan	NCT04522089
West China Hospital of Sichuan University	Protein	China	NCT04470609
Sanofi; GSK	Protein	··	NCT04537208
Merck; Pasteur Institute	Measles vector	France	NCT04497298
Research Institute for Biological Safety Problems	Inactivated virus (QazCovid)	Kazakhstan	NCT04530357
Themis; Merck; University of Pittsburgh Center for Vaccine Research	Vesicular stomatitis virus vectored (COVID-19–101)	Belgium; France	NCT04497298
Symvivo	Oral (bacTRL-Spike)	USA; Canada	NCT04334980
Phase 1 and phase 2 trials			
Imperial College London; Morningside Ventures	Self-amplifying RNA	UK	..
AnGes; Osaka University; Takara Bio	DNA (AG0302-COVID19)	Japan	NCT0452708; NCT04463472
Arcturus; Duke-NUS Medical School	mRNA (LUNAR-COV19)	Singapore	NCT04480957
Johnson & Johnson; Beth Israel Deaconess Medical Center	Adenovirus serotype 26 vector (Ad26.COV2-S)	USA	NCT04436276
Novavax	Nanoparticle (NVX-CoV2373)	USA; South Africa	NCT04533399
Finlay Vaccine Institute	Protein (Soberana 1)	Cuba	··
Vector Institute	Peptide (EpiVacCorona)	Russia	NCT04527575
Bharat Biotech; Indian Council of Medical Research; National Institute of Virology	Inactivated virus (Covaxin)	India	NCT04471519
Anhui Zhifei Longcom Biopharmaceutical; Institute of Microbiology of the Chinese Academy of Sciences	Protein	China	··
Zydus Cadila	DNA (ZyCoV-D)	India	..
Curevac	mRNA (CVnCoV)	Germany, Belgium	NCT04449276, NCT04515147

7.1 Vaccine candidates against SARS-CoV-2 in phase 3 clinical trial

To date, the FDA has issued an Emergency Use Authorization (EUA) for the Moderna, Pfizer-BioNTech, and Janssen COVID-19 vaccines. Several other COVID-19 vaccine candidates remain in development (Table 5).

Table 5: COVID-19 vaccine in phase III clinical trials (Poland GA et al., 2020).

Vaccine Developers	Vaccine type	Location	Trial number
AstraZeneca; University of Oxford (30 000 participants)	Chimpanzee adenovirus (ChAdOx1/AXD1222)	UK; India; Brazil, South Africa; USA	
Moderna; National Institutes of Health (30 000 participants)	RNA (mRNA-1273)	USA	NCT04470427
Pfizer; BioNTech (44,000 participants)	RNA (BNT162b1 and BNT162b2)	USA	NCT04368728
The Janssen Pharmaceutical Companies of Johnson & Johnson (60 000 participants)	Adenovirus serotype 26 vector (Ad26.COV2.S)	USA; Argentina; Brazil; Chile; Columbia; Mexico; Peru; Philippines; South Africa; Ukraine	NCT04505722
The Gamaleya National Research Centre for Epidemiology and Microbiology; Academy of Military Medical Sciences (40,000 participants)	Adenovirus serotype 5 vector and adenovirus serotype 26 vector (Sputnik V)	Russia	NCT04530396
CanSino Biologics; Academy of Military Medical Sciences (40,000 participants)	Adenovirus serotype 5 vector (Ad5CoV)	China; Pakistan	NCT04526990
Sinovac Biotech (9000 participants)	Inactivated virus (CoronaVac)	Brazil; Indonesia	..
Sinopharm; Wuhan Institute of Biological Products (21,000 participants)	Inactivated virus	The United Arab Emirates; Bahrain; Peru; Morocco; Argentina; Jordan	..
Sinopharm; Beijing Institute of Biological Products (5000 participants)	Inactivated virus (BBIBP-CorV)	The United Arab Emirates	..

7.1.1 Inactivated virus vaccines

i. CoronaVac (Sinovac Research and Development Co.)

This vaccine (CoronaVac) is a chemically inactivated, whole-virus preparation administered in a two-dose regimen (at day 0 and day 28) and was granted an emergency use authorisation by Chinese authorities in July, 2020, before the initiation of phase 3 studies. This authorisation reportedly resulted in nearly 90% of company employees being immunised with the vaccine (Wee S-L et al., 2020). No serious adverse events were reported. The vaccine elicited anti-RBD antibodies, as measured by ELISA, and neutralising antibodies 14 days after the second dose of vaccine in 92·4% of individuals receiving the vaccine at 0 and 14 days, and in 97·4% of those receiving the vaccine at 0 and 28 days. Importantly, neutralising antibody responses were significantly higher in younger adults (aged 18–39 years) than in older adults (aged 40–59 years), and stronger responses were noted in participants given the second dose on day 28 than in those given the second dose on day 14. A phase 3 trial has been launched in Brazil and Indonesia, with the trial in Brazil aiming to enrol 9000 health-care personnel.

ii. BBIBP-CorV (Beijing Institute of Biotechnology/China National Biotech Group-Sinopharm)

The inactivated virus vaccine candidate developed by Sinopharm is the result of their collaboration with the Beijing Institute of Biological Products. 2.3.3. BBIBP-CorV was developed by β-propiolactone-mediated inactivation of the 19nCoV-CDC-Tan-HB02 strain SARSCoV-2 that was replicated in Vero cells and adjuvanted with aluminium hydroxide (Xia S et al., 2021). Aluminium hydroxide activates the NLRP3 receptor subunit of the inflammasome and promotes the secretion of high-levels of inflammasome-derived IL-1β and IL-18, thus activating proinflammatory mechanisms of the immune system (He P et al., 2015). Preclinical studies on animal models showed that the aluminium hydroxide-adjuvanted vaccine candidate induced the production of high levels of neutralizing antibodies titers against SARS-CoV-2 as calculated by microtitration experiments. A phase 3 clinical trial began in July, 2020, and plans to enrol 21,000 participants in the United Arab Emirates, Bahrain, Peru, Morocco, Argentina, and Jordan. In late August, 2020, Sinopharm researchers revealed that they had already begun to administer the vaccine to health-care personnel and groups at high risk of becoming infected.

7.1.2 Protein subunit vaccines

i. NVX-CoV2373 (Novavax)

Similar to inactivated pathogen vaccines, protein subunit candidates usually exhibit an extremely favorable safety profile but require multiple boost doses and elicit low grade cellular responses. Maryland-based Novavax has developed a prefusion full-length recombinant SARS-CoV-2 S glycoprotein nanoparticle expressed in a baculovirus-Sf9 system and is administered with an adjuvant named Matrix M1. Saponin based Matrix M1 adjuvant is used precisely to tackle the absence of cell mediated immune responses that characterize protein subunit vaccines (He P at al., 2015). Matrix M-adjuvanted NVX-CoV2373 was first investigated in animal models, such as rats and baboons to assess immunogenicity. Indeed, addition of the adjuvant was found to significantly enhance antibody production in immunized BALB/c mice and induce strong T-cell responses that exhibited a Th1-skewed phenotype. Administration of a two-dose regimen of Matrix M adjuvanted NVX-CoV2373 elicited high titer antibodies that were shown to efficiently neutralize in vitro the cytopathic effects of SARS-CoV-2 on Vero E6 cells and also to prevent the infection of mice transfected to express the human ACE2 receptor with SARSCoV-2. Moreover, these results were replicated in olive baboons receiving intramuscularly two doses of Matrix M-adjuvanted NVXCoV2373 with an interval of 3 weeks (Tian JH et al., 2021). On September 23rd, Novavax launched a Phase 3 trial that aims to enrol up to 9000 volunteers in the United Kingdom and is planning to expand it in the US, India, and other countries.

ii. ZF2001 (Anhui Zhifei Longcom Biopharmaceutical/Chinese Academy of Medical Sciences)

The latest subunit vaccine candidate to enter Phase 3 clinical studies is the adjuvanted RBD-dimeric antigen designed by Anhui Zhifei Longcom Biopharmaceutical and the Institute of Microbiology of the Chinese Academy of Medical Sciences. Phase 3 clinical study was launched on December and will be initially carried out in China and Uzbekistan while Indonesia, Pakistan and Ecuador will follow as study sites (Clinical Trial Identifier: NCT04646590 and Registration Number: ChiCTR2000040153). The design of the study involves recruitment of 22,000 volunteers from China and 7000 subjects outside China for a total of 29,000 volunteers. There are still no published results on this candidate, however data from its Phase 2 placebo-controlled clinical trial (Clinical Trial Identifier: NCT04466085) conducted on a total of 900 participants ranging from 18 to 59 years old suggest that a 2 or 3 dose regimen is evaluated. Each immunization will be separated by the next by 4 weeks (China Daily, 2020).

7.1.3 Nucleic acid vaccines

7.1.3.a mRNA vaccines

i. mRNA-1273 (Moderna/US NIAID)

Moderna's COVID-19 vaccine is an mRNA vaccine that has been shown to be highly effective in preventing symptomatic COVID-19 disease. The vaccine, mRNA-1273, received emergency use authorization (EUA) from FDA in December 2020 for use in individuals 18 years of age and older, making it the second COVID-19 vaccine authorized in the United States. The vaccine is based on an mRNA molecule that contains the information for the synthesis of the stabilized prefusion form of the SARS-CoV-2 S protein encapsulated in a lipid nanoparticle vector that enhances uptake by host immune cells. The administered mRNA uses the host cell transcription and translation machinery to produce the viral antigen that is afterward presented in T lymphocytes and is also directly recognized by B cells of the host, thereby initiating an adaptive immune response directed against the S protein of the virus (Corbett KS et al., 2020). The Moderna vaccination series consists of 2 intramuscular doses, 0.5 mL each, given 4 weeks apart. Second doses administered within a grace period of ≤4 days from the recommended date for the second dose are considered valid; however, doses administered earlier do not need to be repeated. One potential issue for vaccine deployment is that a storage temperature of –20°C is required (Jackson LA et al., 2020).

ii. mRNA-BNT162b2/Comirnaty (Pfizer/BioNTech/Fosun Pharma)

Pfizer-BioNTech's COVID-19 vaccine is an mRNA vaccine that has been shown to be highly effective in preventing symptomatic COVID-19 disease. The vaccine, BNT162b2, received emergency use authorization (EUA) from the U.S. Food and Drug Administration in December 2020 for use in individuals 16 years of age and older, making it the first COVID-19 vaccine authorized in the United States. Preliminary data in non-human primate models revealed that immunization of BALB/c mice with candidate BNT162b2 induced strong humoral and cellular anti-SARS-CoV-2 responses characterized by high titers of specific neutralizing antibodies and activation of $CD8^+$ and $CD4^+$ T lymphocytes that exhibited a Th1 skewed phenotype. Neutralizing antibody levels were assessed with a VSV-based GFP-encoding vector that had been pseudo-typed to present the SARS-CoV-2 S protein on its envelope. Results from Phase 1 randomized placebo-controlled clinical trials showed that BNT162b2 generates minimum side effects both in younger and older participants (Annette B. et al., 2020). Also, two different candidates were evaluated in these trials, namely BNT162b1 and BNT162b2. Both candidates induced the production of similarly high dose-dependent neutralizing antibody titers against SARS-CoV-2 in the inoculated participants. Indeed, the neutralizing antibody titers were higher or equal to SARS-CoV-2 convalescent sera. The Pfizer-BioNTech COVID-19 vaccination series consists of 2 intramuscular doses given 3 weeks apart.

Second doses administered within a grace period of ≤4 days from the recommended date for the second dose are considered valid; however, doses administered earlier do not need to be repeated. BNT162b2 requires storage at –80°C, a fact that could pose logistical problems (Mulligan MJ et al., 2020).

7.1.3.b DNA vaccines

i. INO-4800 (Inovio/International Vaccine Institute)

Although Pennsylvania-based company Inovio has not yet entered officially Phase 3 trials their candidate is the most advanced SARS-CoV-2 DNA vaccine so far. Inovio Pharmaceuticals has developed several experimental DNA-based vaccines which are administered intradermally with the aid of a portable device called 'Cellectra 2000' that delivers a small electric pulse allowing for efficient cellular and nuclear uptake of the DNA molecules through an electroporation mechanism. Their candidate is a two-dose vaccine (INOVIO, 2020).

7.1.4 Replication-defective viral vector vaccines

i. Ad5-nCoV (CanSino Biological/Beijing Institute of Biotechnology/Academy of Military Medical Sciences)

The Chinese company CanSino Biologics in collaboration with the Institute of Biology of China's Academy of Military Medical Sciences developed a candidate using human adenovirus serotype 5 vector (Ad5) to deliver the information that codifies for SARS-CoV-2 full-length S protein into host cells. Ad5 is the main adenoviral serotype in humans, meaning that a significant percentage of individuals may have recent contact, and thus, pre-existing immunity against the viral vector that could hamper robust immune responses against the presented antigen as well.

This candidate vaccine was tested in a phase 1 clinical trial of 108 healthy adults aged 18–60 years. Neutralising antibody titres increased by at least four times from baseline in 11 (31%) of 36 participants in the middle dose group at day 14 and in 18 (50%) at day 28, and in 15 (42%) of 36 participants in the high-dose group at day 14 and in 27 (75%) at day 28 (Zhu FC et al.,2020). The phase 3 trial includes 40 000 participants aged 18 years and older and is underway in Pakistan and China. Information on storage conditions has not yet been released for this vaccine, but storage conditions are likely to be similar to those of other vaccines based on adenovirus vectors and might involve either refrigeration or storage at –20°C.

ii. AZD1222 (AstraZeneca/Oxford University)

Oxford University (Oxford, UK) and AstraZeneca have developed a chimpanzee adenovirus-vectored investigational vaccine (ChAdOx1/AZD1222) encoding the spike glycoprotein of SARS-CoV-2 (van Doremalen N et al., 2020). The vaccine showed both immunogenicity and protective efficacy in non-human primates given a prime-boost vaccination schedule. A phase 1/2 trial with 543 individuals receiving the AZD1222 vaccine tested a prime ($5{\cdot}0 \times 10^{10}$ viral particles) and a prime-boost ($2{\cdot}5 \times 10^{10}$ or $5{\cdot}0 \times 10^{10}$ viral particles) schedule (Pedro M Folegatti et al., 2020). The study showed the induction of humoral responses, characterised by anti-spike glycoprotein IgG and neutralizing antibodies, and IFNγ T-cell responses in most recipients after the first dose of vaccine and an additional increase in humoral immune outcomes after the second dose of vaccine. Humoral immune outcomes in vaccine recipients were similar to those observed in convalescent plasma from patients who had recovered from COVID-19. A significant benefit of Oxford-AstraZeneca's COVID-19 vaccine over the Moderna and Pfizer COVID-19 vaccines is that it can be stored and distributed at 2-8°C.

iii. Gam-COVID-Vac/Sputnik V (Gamaleya Research Institute/Health Ministry of the Russian Federation/Acellena Contract Drug Research and Development)

The Gamaleya National Research Centre for Epidemiology and Microbiology have published the results of two phase 1/2 clinical trials of their COVID-19 vaccine consisting of recombinant adenovirus serotype 26 (rAd26) vector and recombinant adenovirus serotype 5 (rAd5) vector, both carrying the gene for the SARS-CoV-2 spike glycoprotein (rAd26-S and rAd5-S) (Logunov DY et al., 2020). These candidate vaccines ($1{\cdot}0 \times 10^{11}$ viral particles per vaccine dose) were tested in 76 healthy individuals aged 18–60 years (38 participants in each study). Local and systemic reactions were mild, and 100% of recipients in seroconverted, with RBD ELISA titres and neutralizing antibody titres equal to or more than titres observed in convalescent plasma from patients who had recovered from COVID-19. $CD4^+$ and $CD8^+$ Th cell immune responses were detected in all volunteers and peaked at day 28 after vaccination. A phase 3 safety and efficacy trial will recruit 40,000 participants from different age and risk groups (Bucci E, 2020).

iv. JNJ-78436735/Ad26.COV2.S (Janssen and Beth Israel Deaconess Medical Center)

Janssen Pharmaceuticals is the vaccine development branch of Johnson & Johnson pharmaceutic. Their candidate is a replicating-defective adenovirus 26 based vector expressing the stabilized pre-fusion S protein of SARS-CoV-2, a method developed a decade ago by researchers of the Beth Israel Deaconess Medical Center. Their main difference from the CanSino vaccine candidate is the adenovirus serotype. As opposed to the ubiquitous Ad5 serotype, very few people have been exposed to the rare Ad26 serotype, therefore, pre-existing immunity against the vector reducing this candidate's immunogenicity is not expected to be a major concern. The second advantage of this candidate is that the dosing schedule involves a single immunization. This candidate vaccine requires storage at 2–8°C (Loftus P, 2020). On November 15th, Janssen informed that they

will initiate a second Phase 3 randomized, double-blind, placebo-controlled clinical trial studying the safety and efficacy of a two-dose regimen of their candidate. The study will involve 30,000 adult participants from Belgium, Colombia, France, Germany, the Philippines, South Africa, Spain, the United Kingdom and the United States that will receive either two doses of the Ad26.COV2. S vaccine candidate or a placebo with a 57-day interval (Johnson & Johnson, 2020).

8. CHALLENGES IN VACCINE DEVELOPMENT

An ideal vaccine should be safe, even in immunocompromised people, should be highly effective and optimally induce 'sterilizing' immunity, should retail immunogenicity despite adverse storage, should be inexpensive, free from toxicity and adverse effects, should give long term protection, should have high thermal stability. Vaccine development is a lengthy, expensive process and many challenges arise during the development, manufacturing, and mass distribution (Ada GL, 1991).

8.1 Time

The major challenge in developing SARS-CoV2 vaccine is the fast tracking of every step in the discovery, development, and evaluation process. Traditional vaccine development can take 15 years or more, starting with a lengthy discovery phase in which vaccines are designed and exploratory preclinical experiments are conducted (Figure 15). This is usually followed by a phase in which more formal preclinical experiments and toxicology studies are performed and in which production processes are developed. During this process an investigational new drug (IND) application is filed and the vaccine candidate then enters phase I, II and III trials. If, when phase III trials are completed, the predetermined end points have been met, a biologics licence application (BLA) is filed, reviewed by regulatory agencies and finally the vaccine is licensed. After that point, large-scale production begins (Krammer F, 2020). However, due to an expedited increase in the number of COVID-19 cases worldwide, vaccine regulatory authorities both at international and national levels are forced to fast track every process of development to meet the world's immediate vaccine requirement. The scientific communities are using multiple approaches to shorten development phase including overlapping clinical phase and using advanced computer-aided and biotechnological tools. Vaccine development for SARS-CoV-2 is following an accelerated timeline. Because of knowledge gained from the initial development of vaccines for SARS-CoV and MERS-CoV, the discovery phase was omitted. Existing processes were adopted, and phase I/II trials were started. Phase III trials were initiated after the interim analysis of phase I/II results, with several clinical trial stages running in parallel. In the meantime, vaccine producers have started the large-scale production of several vaccine candidates, at risk.

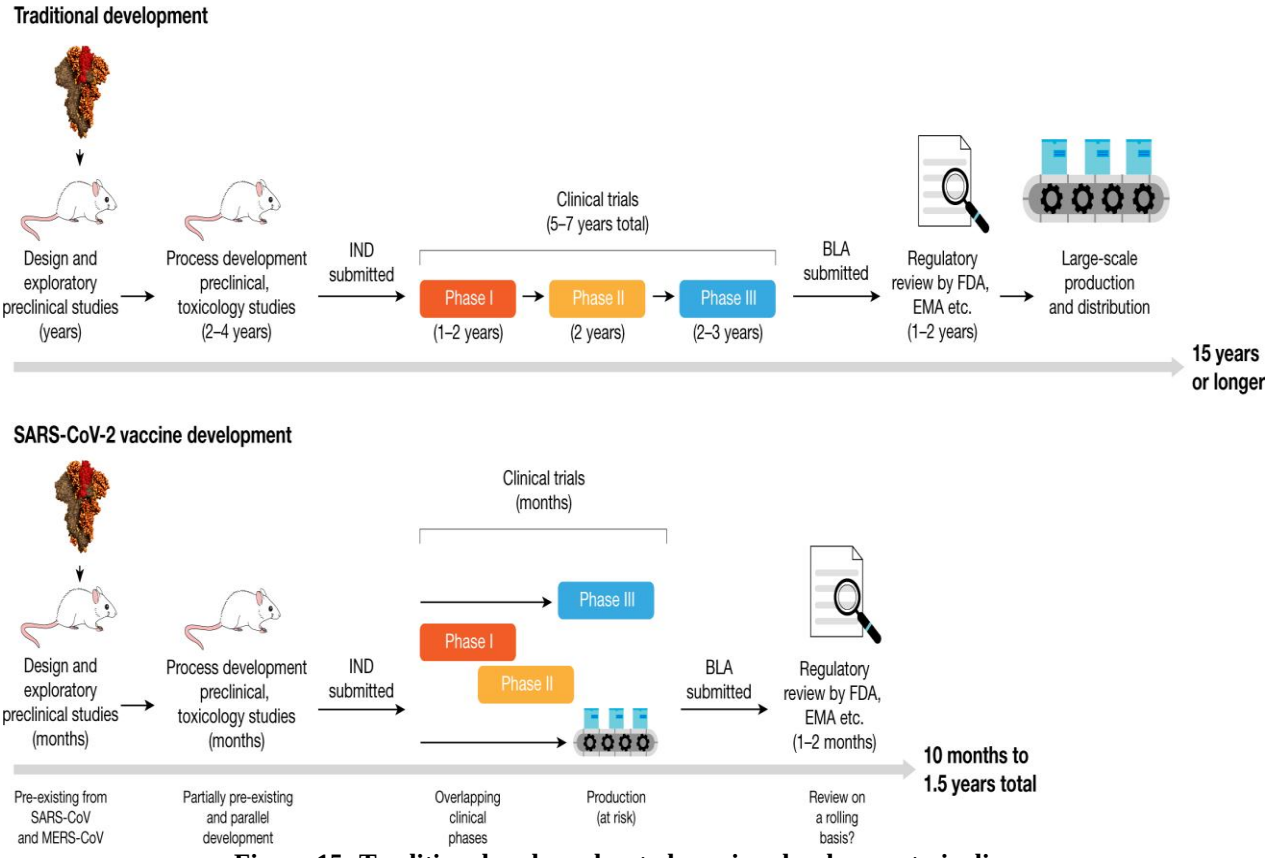


Figure 15: Traditional and accelerated vaccine-development pipelines.

8.2 Toxicity and adverse effects

The fast-tracking of vaccine development processes highten the risk of increased side effects. There is an immense possibility that some essential data might go missing or unnoticed at this accelerated speed of development. Researchers are concerned about the risk to public health if any important evaluation goes under notice. The use of animal models as a requirement of preclinical evaluation is usually needed to assess the safety and access risk associated with the new vaccine candidates or combination vaccines before starting first-in-human trials. Thorough pre-clinical and laboratory testing have to go in compliance with good laboratory practice guidelines and with national guidelines on animal experimentations. These studies are also necessary to establish characteristics (physical, chemical, and biological) of the vaccine candidates. Some research groups have omitted these essential animal testing studies in the face of global health crisis while others are running parallel pre-clinical and first-in-human trials. This break from the usual protocol is worrisome and a challenge that requires prime attention. Note that the translation of animal studies to humans may not 100% with regards to toxicity studies. However, some animal models such as the mice, ferrets, syrian hamster, and rhesus macaques are demonstrating symptoms similar to humans upon exposure to SARS-CoV-2 (Deb B et al., 2020).

Safety assessment of very recent technologies like DNA, RNA, VLP's is to be given more importance as even if there is evidence of safety and efficacy, there are very few/no vaccine so far licensed and used in a large population. Serious adverse events and allergies were previously reported due to protein, non-protein impurities found in vaccines therefore thorough quality check during the manufacturing process is mandatory before distribution. A lamentable occurrence had occurred on account of polio immunization in 1955 because the cycle of inactivating the live virus was flawed. There were reports of paralysis and within a month the mass immunization program against polio was deserted. It was later uncovered that the vaccine had resulted in causing 40,000 instances of polio, leaving 200 youngsters with differing degrees of paralysis and several deaths (Fitzpatrick M, 2006). Therefore manufacturing methodology has to be extremely audited and validated; manufacturers should be extremely meticulous in producing large quantities of vaccine doses. This will be challenging during the present race to license the first SARS-CoV2 vaccine and mass-produce a large number of doses.

8.3 Mutations

In the early pandemic situation, there was concern among the scientific community over mutations arising in SARS-CoV-2. However recent studies have indicated no cause for concern. The outcomes of phylogenetic examination of various SARS-CoV-2 strains procured from various nations showed that all the glycoproteins of various strains of SARS-CoV-2, obtained from various nations were strongly related to each other; hence antibody structured against one strain would be successful against the various strains of SARS-CoV-2 from various nations. Nevertheless, it is essential to continuously monitor genomic sequence given the knowledge of previous experience on virus mutation rate (Duffy S, 2018).

8.4 Long term protection

Ideally, vaccination should provide long term protection. However, immunization induced resistance blurs after some time and the loss of protection varies with every disease (John Cohen, 2019). Two doses of inactivated polio antibody (IPV) are 90 % effective or more against polio and three doses are 99 %–100 % effective and the duration of protection lasts for several years to decades (CDC, 2018). Most promising SARS-CoV-2 vaccine candidates in clinical trials require booster doses. It is too early to say any of it provides long term protection. Reinfection is another major aspect affecting the protection period. A very recent study has confirmed reinfection with genomic evidence. It was concluded that SARS-CoV-2 might flow among the human populace regardless of crowd insusceptibility on account of general infection or immunization. Additional monitoring of patients with reinfection will help optimized vaccine design against SARS-CoV-2 (To KK et al., 2020).

8.5 Antibody-dependent enhancement (ADE)

Vaccination induces humoral and cellular immune response in immunized individuals. In the normal condition, when the homologous virus enters an immunized body, it will be neutralized or cleared by vaccine- induced neutralizing antibodies (Abs) or specific T cells, respectively. In the context of vaccine-associated disease enhancement, vaccines mainly induce non- neutralizing Abs or low titres of neutralizing Abs (suboptimal concentration) or type 2 T helper cell (TH2 cell)- biased T cell responses (Figure 16). When these vaccinated individuals are challenged by homotypic or heterotypic serotype viruses, the antibodies will immediately recognize the viruses and mediate antibody-dependent disease exacerbation in two ways. First, virus–antibody complexes might enter F_c receptor (F_cR)-bearing cells, such as dendritic cells and monocytes, by F_cR- mediated internalization, which is termed 'antibody- dependent enhancement' (ADE). For viruses with innate tropism for F_cR- bearing cells, such as dengue virus, ADE will result in higher viral loads than in conditions without antibodies. After entry, the virus, no matter whether it replicates or does not replicate, may activate a harmful immune response, resulting in the release of proinflammatory cytokines (Tirado SM et al., 2003).

ADE of ailment is an overall worry for the development of immunizations and treatments since it possibly intensifies the infection or triggers dangerous

immunopathology (Arvin AM et al., 2020). A serious ADE was seen in the case of the dengue vaccine. The rate of hospitalization was increased in vaccinated children than in non-vaccinated children. It was discovered that the antibody imitated the primary infection and that a reduction in the immunity presented a few youngsters to the danger of ADE in case of a subsequent secondary infection. ADE is believed to be liable for causing COVID-19. So far there are none in-vitro, in-vivo, or clinical proof of ADE happening in COVID-19 patients nonetheless, ADE may represent some serious results during the regular course of the illness (Negro F, 2020). Careful monitoring of ADE over several years post-vaccination is necessary especially during the mass vaccination program as ADE is evident when enough people have been vaccinated.

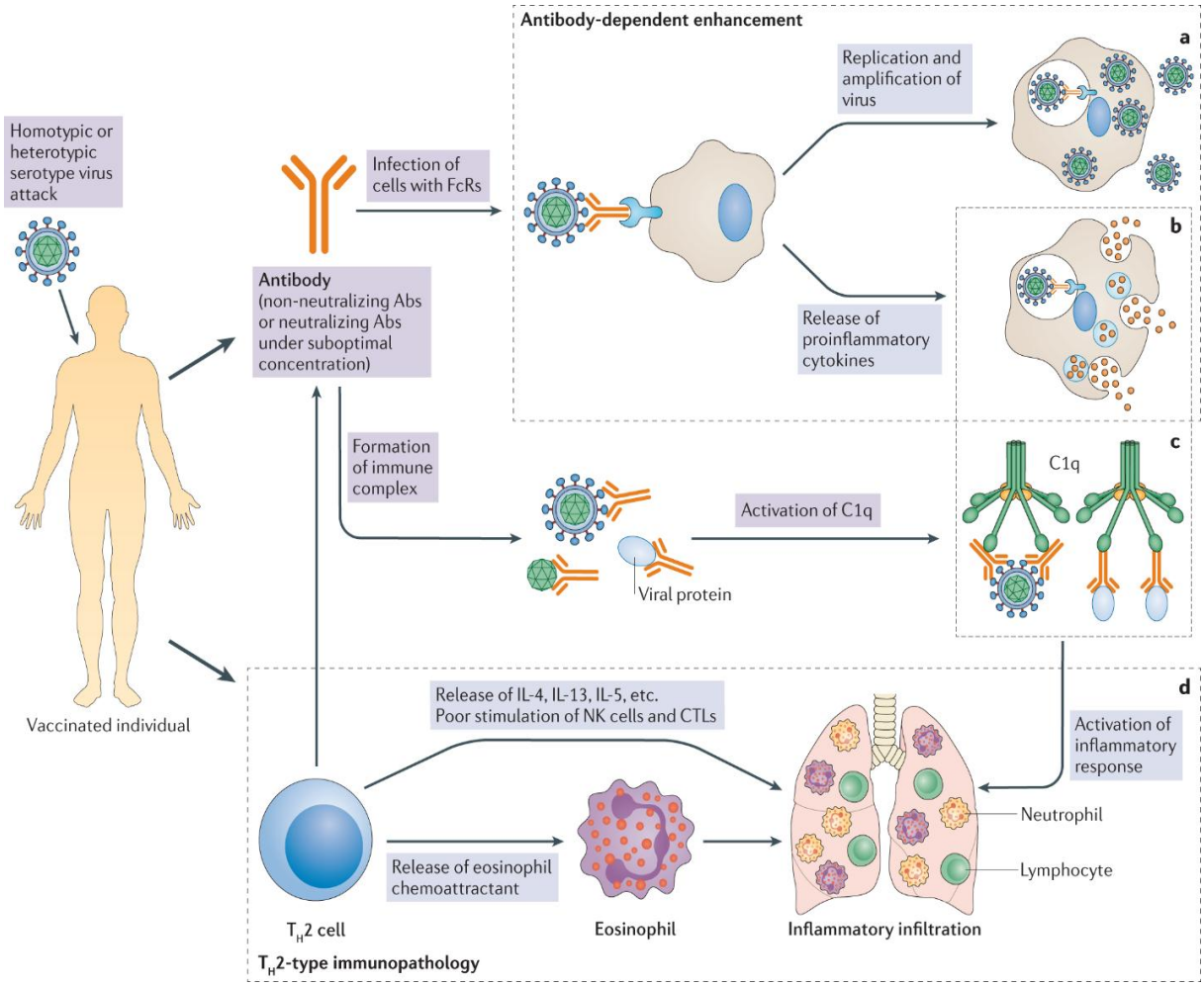


Figure 16: Mechanisms of vaccine-associated disease enhancement. a | ADE causes an increased viral replication or b | may activate the release of proinflammatory cytokines. c | Aside from ADE, antibody–antigen complexes can stimulate the complement pathway through activation of the C1q pathway, thus further strengthening the inflammatory responses. d | Vaccine- associated disease enhancement can also involve a TH2 cell- biased immune response. The activated TH2 cells contribute to the activation of antibody production. However, they release interleukin-4 (IL-4), IL-13 and IL-5, as well as eosinophil chemoattractant, thus resulting in eosinophil infiltration and proinflammatory cytokine production in the lung. Natural killer (NK) cells and $CD8^+$ cytotoxic T lymphocytes (CTLs) are poorly stimulated in TH2 cell- skewed immune responses. The exaggerated cytokine release, activation of the complement pathway and the excessive mobilization of eosinophils all contribute to the infiltration of the lung by eosinophils, neutrophils and lymphocytes, and production of inflammatory cytokines, leading to acute lung injury or acute respiratory distress syndrome.

8.6 Cost

Even if a single vaccine is proven safe and efficacious, large scale manufacturing and distribution will be challenging especially if vaccine candidate involves novel technologies as very few manufacturing plant have previous experiences in mass production. The establishment will have to comply with the GLP guidelines for the particular vaccine candidate. Setting up new premises and infrastructures for vaccine production which is meeting complete quality guidelines will have cost involving. Also, in the current global rush to develop a vaccine, there is a possibility that this very crucial compliance step might miss adequate attention; posing a potential danger. The challenge is to vaccinate

the entire world population. Experts worry that this might be physically difficult to achieve owing to resource scarcity. Also, there should be production balanced against the need for other vaccines. The kind of infrastructure needed for production will depend on the type of vaccine (To KK et al., 2020). The world's governments and companies need to invest enough money so that vaccines can be made quickly available. Financial losses may also occur if the COVID-19 pandemic is ended before development phases are completed as experienced from previous epidemics like SARS and MERS.

9. CONCLUSIONS AND PROSPECTS

The rapid spread of severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2) has elicited an equally rapid response aiming to develop a COVID-19 vaccine. There are more questions than answers for the newly identified virus, including the etiology, epidemiology, structural basis, mechanism of pathogenesis, pathological immune response, etc. Particularly, the cellular and humoral immune response of the host in response to the virus infection that are essential for vaccine design, remains unclear. All these aspects need to be addressed by basic research in the near future for successful vaccine development. Countries all over the world, regardless of political ideologies, can unite and work together to achieve fast and successful COVID-19 vaccine development in the near future. More studies are required urgently to reach the most successful vaccine candidate in order to minimize the growing number of COVID-19 cases. Recently, more and more countries and R&D institutions announced their program on vaccine development against SARS-CoV-2.

However, vaccine development has its own rules. After vaccine design and preparation, it will undergo efficacy and safety evaluation, quality standard establishment before entering clinical trials. Post-licensure vaccine safety assessment is a long-term assessment of adverse event occurring post-vaccination. Keeping entire hope on a vaccine to end the pandemic should not be encouraged. Since the vaccine alone cannot combat the pandemic, modern preventive social strategies are needed so that the world can battle the present pandemic and be able to face another pandemic if it occurs in the future. According to WHO, world leaders and the public must follow and come up with a novel social measure to reduce viral concentrates within the human populace.

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