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Strategies of SARS-CoV-2 Vaccine Production Using Nucleic Acid Based Vaccines and Conventional Methods

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DEDICATED TO

To my mother Mrs. Bridget Ekpela who believed in me and supported me since the start of this journey, who played the role ofboth a mother and a father in my life, for her constant prayers, advices, encouragement to persuade me to not give up when things were hard.

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Abstract

SARS-CoV-2 is the causative agent of the disease COVID-19 which emerged in 2019 and leads to a global pandemic. SARS-CoV-2 has genetic similarities with other human coronaviruses (MERS-CoV and SARS-CoV-1). Vaccination is the most effective method in preventing deaths, hospitalization, and the transmission of the infection. Conventional vaccine development usually lasts 10 to 15 years. Considering SARS-CoV-2 is highly contagious and highly transmissible, several innovative vaccines were developed in a short period to help combat this pandemic:

Vaccines are based on the viral genome and the whole structure as well to induce the host immune system. SARS-CoV-2 is cultured in lineage cells like VERO cells to produce inactivated and live attenuated vaccines, which are subsequently rendered inactive through chemical processes. Using RNA as mRNA and DNA as plasmids, genetic vaccinations are created.

The Spike protein is a potential element in SARS-CoV-2 vaccine designing. The viral recombinant spike protein is difficult to produce due to its size and its membrane fusion protein which is metastable and heavily glycolated. Therefore, the expression of SARS-CoV-2 recombinant protein fragments in *E.coli* is an effective approach in recombinant protein production. All vaccines were tested *in vivo* on animals and humans to regulate their safety and effectiveness. Since SARS-CoV-2 mutates, new strategies are required to generate new vaccines, while keeping all the preventive majors that have been implanted by governments and WHO.

Key words: SARS-Cov-2, covid-19, pandemic, immune system, spike protein, vaccines platforms.

ملخص

يعتبر فيروس المتلازمة التنفسية الحادة الوخيمة كورونا 2 (سارس-كوف-2) العامل المسبب لمرض كوفيد-19 الذي ظهر في عام 2019 والذي أدى إلى جائحة عالمية. لدى سارس-كوف-2 أوجه تشابه جينية مع فيروسات كورونا البشرية الأخرى (المتلازمة التنفسية الشرق أوسطية **MERS**) و(سارس-كوف-1) .

اللقاح هو الطريقة الأكثر فاعلية في منع الوفيات والاستشفاء وانتقال العدوى. عادة ما يستغرق تطوير اللقاح الكلاسيكي من 10 إلى 15 عامًا. بالنظر إلى أن (سارس-كوف-2) شديد العدوى وسريع الانتقال، فقد تم تطوير العديد من اللقاحات في فترة زمنية قصيرة للمساعدة في مكافحة هذا الوباء.

تعتمد اللقاحات على الجينوم الفيروسي وعلى بنيته الكاملة، أيضًا لتحفيز الجهاز المناعي المضيف. يتم إنتاج اللقاحات الحية الموهنة او المعطلة عن طريق زراعة سارس-كوف-2 في السلالات الخلوية مثل خلايا فيرو للحصول على منتجات فيروسية عالية التركيز بحيث يتم تعطيلها عن طريق العلاجات الكيميائية. يتم إنتاج اللقاحات الجينية باستخدام دي إن أي (مثل البلازميدات) و ار إن أي(الحمض النووي الريبوزي المرسال) .

يعتبر البروتين التاجي عنصرًا محتملاً في تصميم لقاح سارس-كوف-2. يصعب إنتاج هذا البروتين الهجين نظرًا لحجمه وبروتين غشاء الاندماج الذي يكون ثابتًا ومغلفًا بشكل كبير. لذلك، فإن التعبير عن البروتين الهجين سارس-كوف-2 في بكتيريا القولون هو نهج فعال في إنتاجه. تم اختبار جميع اللقاحات في الجسم الحي على الحيوانات والإنسان لتأكد من سلامتها وفعاليتها. منذ تحور سارس-كوف-2، هناك حاجة إلى استراتيجيات جديدة لإنتاج لقاحات جديدة، مع الحفاظ على جميع الإجراءات الوقائية المتخذة من قبل مختلف المنظمات الصحية الدولية من اجل التقليص من العدوى .

الكلمات المفتاحية: سارس-كوف-2، كوفيد-19، الجائحة، جهاز المناعة، بروتين التاجي، منصات اللقاحات.

Résumé

Le SRAS-CoV-2 est l'agent causal de la maladie COVID-19 qui a émergé en 2019 eta conduit à une pandémie mondiale. Le SRAS-CoV-2 présente des similitudes génétiques avec d'autres coronavirus humains (MERS-CoV et SRAS-CoV-1). Lavaccination est la méthode la plus efficace pour prévenir les décès, les hospitalisationset la transmission de l'infection. Le développement d'un vaccin classique dure habituellement de 10 à 15 ans. Le SRAS-CoV-2 étant très contagieux et hautement transmissible, plusieurs vaccins innovants ont été développés en peu de temps pour aider à combattre cette pandémie :

Les vaccins sont basés sur le génome viral ainsi que sur l'ensemble de sa structure pour induire le système immunitaire de l'hôte. Le SRAS-CoV-2 est cultivé dans des cellules de lignée comme les cellules VERO pour produire des vaccins inactivés et vivants atténués, qui sont ensuite rendus inactifs par des procédés chimiques. Les vaccins génétiques sont créés en utilisant l'ARN comme ARNm et l'ADN comme plasmide.

La protéine Spike est un élément potentiel dans la conception du vaccin contre le SRAS-CoV-2. La protéine spike recombinante virale est difficile à produire en raison de sa taille moléculaire et de sa protéine de fusion membranaire qui est métastable et fortement glycolée. Par conséquent, l'expression de fragments de protéines recombinantes du SRAS-CoV-2 dans E. coli est une approche efficace pour la production de protéines recombinantes. Tous les vaccins ont été testés in vivo sur des animaux et des humains afin de vérifier leur sécurité et leur efficacité. Comme le SRAS-CoV-2 mute, de nouvelles stratégies sont nécessaires pour générer de nouveaux vaccins, tout en maintenant les mesures préventives qui ont été mises en place par les gouvernements et l'OMS.

Mots clés : SARS-Cov-2, covid-19, pandémie, système immunitaire, protéine spike, plateformes de vaccins.

List of abbreviation

ADE: Antibody Dependant Enchanchement

CD: Connecter Domain

CH: Center helix

COPD: Chronic Obstructive Pulmonary Disease

CT: Cytoplasmic tail

CXXL10: C-X-C Motif chemokine 10

FP: Fusion peptide

hACE: Human angiotensin converting enzyme

HR: Heptard repeat

ICTV: International Committee of taxonomy of viruses

MERS-CoV-2: Middle East Respiratory Syndrome

MHC: Major Histocopatibility Complex

NGS: Metagenomic next generational sequencing

Nsp: Non-structural proteins

NTD: N-terminal Domain

RBD: Receptor-binding domain

RNP: Ribonucleoprotein

RT-PCR: Real Time PCR

SARS-CoV-2: Server Respiratory Syndrome

TD: Transmembrane Domain

TMPSS2: Transmembrane serine protease

WHO: World Health Organization

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Introduction

Pathogens including viruses are not new to human understanding. Human races have been combating viruses and bacterial pathogens for centuries. Some of the significant human viruses that emerged over time include, swine flu (H1N1) in 2009, Ebola in the western part of Africa in 2014, Zika virus in 2016, Ebola in Congo in 2016 and influenza; the flu virus thatcauses common cold (Ganesh *et al., 2021).*

In 2019, there was a viral outbreak in the city of Wuhan, the capital of Hubei Province in China, which was highly contagious and deadly disease. Patients of the novel virus in Hubei were reported to have life-threatening symptoms such as shortage of breath, coughing, severe chest pain and more. This virus was transmitted between humans with over 10 million confirmed cases and 500,000 deaths worldwide by the end of June 2020. Considering the high transmission rate of this novel virus, the World Health Organization (WHO) announced it to be a global concern and named the disease caused by this virus COVID-19 on 30 January 2019. While the international committee of taxonomy of viruses (ICTV) named the virus severe respiratory acute syndrome coronavirus 2 (SARS-CoV-2) on February 2019 (Ben *et al.*, 2021). SARS-CoV-2 which is the causative of COVID-19 was declared a global pandemic by WHO on March 11, 2020 (Kumar *et al.*, 2021), the most challenging pandemic of the century.

This virus was able to adapt and mutate easily (Hirabara *et al.*, 2022). As a result, Covid- 19 is continuing to spread around the world, with more than 530 million confirmed cases and more than six million deaths reported across almost 200 countries (Johns Hopkins University, 2022).

Several SARS-CoV-2 vaccine platforms were developed. These platforms are based on traditional method such as inactivated or live virus vaccines (PiCoVacc), and new methods such as recombinant protein and viral vector vaccines, RNA (mRNA 1273) and DNA vaccines (GLS-5300 (INO-4700) (Krammer, 2020; Qiang *et al.*, 2020; Takehiro *et al.*, 2021; Yen-The *et al.*, 2020).

From the mentioned facts, the need for vaccine development to decelerate SARS-CoV-2 is urgently required.

The aim of this study is to investigate the principles and methods used by pharmaceutical industries to produce vaccines candidates. How DNA, RNA and inactivated whole SARS-CoV-2 is used in vaccine development.

Chapter I focuses on human different emerged human coronaviruses leading to epidemics and or pandemic. It comprises SARS-CoV-2 target cells, mechanism and receptors used in viral entry into host cells causing infection. It also illustrates effective approach to decelerate SARS-CoV-2 and the human immune response to the viral infection.

Chapter II demonstrates the technologies used in the production of different types of vaccines. It explains how plasmid vectors are generated and amplified in bacterial cultures, to produce plasmid-based DNA vaccine at a laboratory scale. The new challenges in combating Covid-19 infections are also cited in this chapter.

CHAPTER I

Chapter I: Coronaviruses

1. Definition

Coronaviruses *(*CoVs*)* are viruses with a crown-like structure when viewed under electronic microscope (Figure 1). Due to this structure, these viruses are named coronaviruses (Gao *et al.*, 2021*)*. CoVs are a family of viruses that causes respiratory and intestinal infection in animals and humans. They cause mild cold and severe pneumonia by infecting the respiratorytract of their host (Ben *et al.*, 2021).

Usually, the clinical manifestation of CoV includes coughing, shortness of breath, flu, and high fever (Li and Chi, 2020).

CoVs are spherical in shape and coated with spike proteins on the surface membrane. They have a larger genome as compared to other RNA viruses (Kumar *et al.*, 2021).

Viruses that belong to the family (Coroviridae) are subdivided into three groups: Group 1, Group 2 and Group 3 (Kumar *et al.*, 2021). The group 1 and group 2 infect mammals, while those in group 3 have birds as their hosts and till date, the viruses of this group have only been isolated from birds (Chamings *et al.*, 2018).

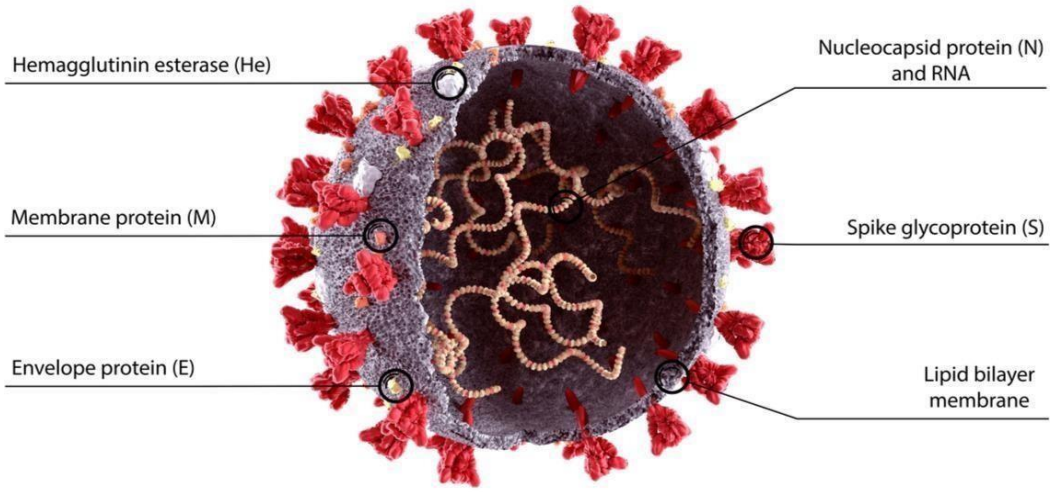

Figure 1: Structure and assembly of SARS-CoV-2 nucleocapsid protein (Liji, 2022)

2. Different classes of human coronaviruses

Among the different human and animal coronaviruses that have been discovered until date, there are seven human coronaviruses identified as shown in table 1. Some of these classes can only cause mild symptoms by infecting the human respiratory tract and these groups include the Human Coronavirus 229E (HCoV-229 E), Human Coronavirus OC43 (HCoV-OC43), Human Coronavirus NL63 (HCoV-NL63) and Human Coronavirus HKU1 (HCoV-HKU1) (Wang *et al.*, 2020). While the rest of the three classes cause severe respiratory diseases including organ failures. These classes of coronaviruses include severe acute respiratory syndrome coronavirus (SARS-CoV*)*, Middle East respiratory syndrome coronavirus (MERS-CoV) and the novel RNA single stranded strain virus known as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) which is the causative agent of COVID-19 (Wang *et al.*, 2020).

Table 1: The seven classes of human coronaviruses (Chi *et al.*, 2019)

Human Coronaviruses	Sickness Cause
HKU1	Mild Respiratory Illness
HCoV-OC43	Mild Respiratory Illness
HCoV-229^{E}	Mild Respiratory Illness
HCoV-NL63	Mild Respiratory Illness
MERS-CoV	Middle East Respiratory Syndrome (MERS)
SARS-CoV	Severe Acute Respiratory Syndrome (SARS)
SARS-CoV-2	COVID-19

2.1 Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

In 2012, there was an outbreak of another human coronavirus known as Middle East Respiratory Syndrome (*MERS-CoV)* in Saudi Arabia that leads to 806 deaths and 2279 infected confirmed cases (Ganesh *et al.*, 2021). Regardless of our basic understanding of these coronaviruses, we were not prepared for the new emerged class of coronavirus *SARS-CoV-2* (Krammer, 2020).

2.2. Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV*)*

The severe acute respiratory syndrome otherwise known as SARS is a virus that is transmitted from bats to humans. SARS became apparent in 2003 (Fong *et al.,* 2020). This virus has 25% fatality rate and is highly transmissible, infecting all age groups (Fong *et al.,* 2020).

Gradually other human and animal viruses were discovered which were named coronaviruses because of their crown-like structure under electronic microscope (Ben Hu, 2021). Among these viruses, seven classes are identified to infect humans (Fang *et al.,* 2016). Till date different viruses have emerged and lead to global pandemics (Kaul, 2021).

In November 2002, there was an outbreak of a human coronavirus in Guangdong of China. The disease was known as severe acute respiratory syndrome (SARS) that spread to 29 countries and lead to 774 deaths and 8,000 infected cases reported within a period of one year. Initially, the clinical manifestations were mild symptoms, but severe conditions were later observed which lead to the (WHO) announcing SARS as a global health concern in 2003. Another outbreak was reported in the year 2004, whereby, patients had clinical symptoms such as cold, difficulty in breathing, coughing, headache, and high fever even though reported cases were minor. Since then, researchers started to develop different strategies to produce *SARS* vaccines (Ganesh *et al.,* 2021).

2.3 SARS-CoV-2

In 2019, there was a viral outbreak in the city of Wuhan, China. Patients of the novel virus were reported to have life-threatening symptoms such as shortage of breath, coughing, severe chest pain and more. The clinical manifestations of the novel virus were similar to that of MERS-CoV and SARS-CoV, but it has features that made it highly contagious (Kumar *et al.,* 2021). This novel virus was traced back to an open seafood, wildlife animals market in Wuhan where it was believed to have started.

Metagenomic next generation sequencing (mNGS) was conducted on patient samples. After which the novel virus was identified as beta coronavirus and similar to SARS-CoV and MERS-CoV. Due to the high transmission rate of this novel virus, it was able to spreadto 34 other cities in China in a duration of one month with new daily reported cases. As a result of this,

Table 2: Clinical comparison between SARS-CoV-1, SARS-CoV-2and MERS-CoV (Pustake *et al.*, 2022)

Features	SARS-CoV-1	SARS-CoV-2	MERS-CoV
Incubation	2-10 days	2-14 days	2-12 days
Fever	100%	34-80%	81-98%
Cough	75-80%	19-57%	57-83%
Chills	15-90%	25%	85%
Myalgia	45-50%	6.5-34%	43%
Headache	20-70%	2.5-38%	20.4%
Dyspnea	35-60%	6-36%	22-72%
Tachypnea	40-75%	30-35%	-
Tachycardia	40-75%	20-27%	-
Hypoxemia	40-75%	40-50%	-
Cachexia	-	35%	-
Malaise	40-75%	56%	38%
Vomiting	35%	2-13%	14-21%
Diarrhea	6-25%	5-21%	19.4-26%
Sore throat	25%	2.5-10%	9.1-14%
Rhinorrhea	15%	5-10%	1.6%
Hemoptysis	-	22.4%	4.3%
Asymptomatic	-	6.5%	-

The WHO announced the emerging virus to be a global concern and named the disease caused by the virus, COVID-19 on January 30, 2019, while the international committee of taxonomy of viruses (ICTV) gave the novel coronavirus the name severe acute respiratory syndrome coronavirus 2 (SARS-COV-2*)* on the 11th of February (Kumar *et al.*, 2021).

SARS-CoV-2 spread to over 216 countries with 733,000 deceased infected individuals within few months from the first reported case (Kumar *et al.,* 2021). After that WHO declared it on 11 March 2020 (Gao *et al.,* 2020).

Researchers have accumulated much information about this novel virus in very short period. In understanding the genomic structure, target cells and life cycle of *SARS- CoV-2*, aids in vaccine development. To stop the spread of this novel virus, vaccines are the only effective major. Until date, there are different vaccine candidates and platforms that have developed *SARS-CoV-2* vaccines. Although, promising, new strategies are still required to be developed against *SARS-CoV-2.* Technique of using the surface structural proteins-based vaccine is an encouraging method to fight *SARS-CoV-2* (Kumar *et al.,* 2021).

2.3.1 Genomic Structure of SARS-CoV-2

SARS-CoV-2 is an RNA genome virus with positive sense, single-stranded that is 29.9 kb in length with 6 or more opening reading frames (ORFs) from the 5′ end to the 3′ end in the order of (ORF1a ORF1b), the Spike protein (S), an envelope (E), a membrane (M) and nuleocapside (N) (Ben and Hua, 2021). ORF1a and ORF1b codes many nonstructural proteins (NSPs) and cover a bigger potion of the virus genome Ayman and Al-Qaaneh. 2021). SARS-CoV-2 has 79% genome similarity with *SARS-CoV* and 50% with the genome of MERS-CoV. About 90 of the amino acids which are found in SARS-CoV-2 are also found in SARS-CoV except that there is no gene than codes for the spike (S) surface protein in the structure of SARS-CoV (Ferns *et al.,* 2021). SARS-CoV-2 genome external membrane is made up of spike proteins in a primarily profusion conformation with a small fraction of a portion of S in its post fusion form (figure 2a). The spike (S) appears as a flexible head on a stalk and can bend or tilt to 90 °c to the membrane, with the majority appearing to tilt or bend less than 50 °C. The flexibility is provided by the hings in the stalk regions (Figure 2b) (Hardenbrook *et al.,* 2022).

The surface of the (S) timer is glycosylated, with each of the Spike monomer containing 22 glycosylated sites. The glycan coat paired with flexibility of SARS-CoV-2 spikes enables them to recognize the host cell surface and binds to the receptor (ACE2) while protecting them from not been neutralized by the host neutralizing antibodies (Hardenbrook *et al.,* 2022).

SARS-CoV-2 outer membrane is made of the membrane (M) protein and the envelope (E) protein (Figure 2b). Inside or within the lumen of the virus is the ribonucleoproteins (RNP) complex which contains the nucleocapsid (N) protein and viral genome that is responsible in packaging the viral RNA with an estimate of 30-35 RNPs per virion (Figure 2b). (Hardenbrook *et al.,* 2022). The viral nonstructural proteins are produced from a self-cleavage of the precursor-polyprotein Pp1a and Pp1ab by *SARS-CoV-2* proteases. PLpro (Nsp3) cleaves three sites that results in free Nsp1-3, the Mpro (Nsp5) cleave the remaining 13 cleavage sites. This allows or permits the nonstructural proteins to perform their role in the host cell ranging from RdRp (Nsp12) and helicase (Nsp13) functions, creating a double membrane vesicle (MVs) for the viral RNA genome replication, transcription and transport (Hardenbrook *et al.,* 2021).

2.3.2 SARS-CoV-2 epidemiology

SARS-CoV-2 is transmitted through droplets by close contact of an infected individual that is not protected. Both symptomatic and asymptomatic patients are sources of SARS-CoV-2 infection. Infection occurs both through direct and indirect contact. Although SARS-CoV-2 is a respiratory tract infection virus, it is not limited to the respiratory tract. Studies have shown that SARS-CoV-2 aerosol transmission is possible. This is confirmed through an investigation done as of the COVID-19 outbreak where patients samples were taken to investigate the aerodynamic nature of SARS-CoV-2. This research was done by measuring the viral RNA in aerosols at two hospitals in Wuhan, which actually indicates the possibility and potential of SARS-CoV-2 been spread via aerosols even though it is not the dominant route of transmission. This indicates that there are chances of airborne transmission in the health care facilities as a result of aerosols which are generated by different medical procedures (Wang *et al.,* 2020).

Studies showed that SARS-CoV-2 can replicate in the human intestinal organoids and intestinal epithelium, enabling SARS-CoV-2 to have the potential to spread through the intestinal tract. According to Sun *et al.* (2020), SARS-CoV-2 was found in COVID-19 patient's urine (Wang *et al.,* 2020).

SARS-CoV-2 infection occurs in all age range and the symptoms differ for all different age group. Middle age populations are most likely to get infected and individuals who are older than 60 years can develop severe pneumonia which can lead to death. SARS-CoV-2 was identified in blood samples, stool specimens and semen.

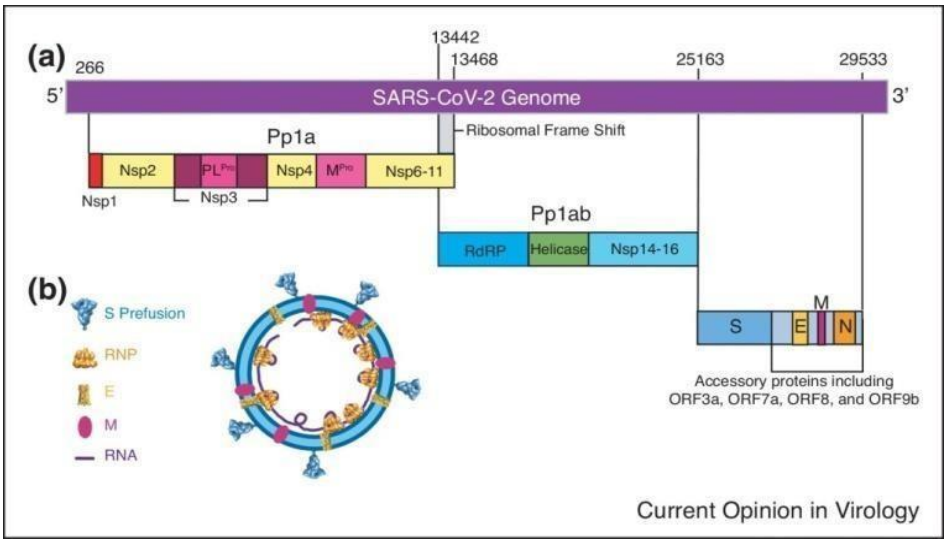

Figure 2: SARS-CoV-2 structural organization (A) shows the general genomic organization of SARS-CoV-2.

Nonstructural proteins Nsp1-16 are expressed as polyproteins Ppla and Pp1ab are Cleaved by internal Mpro and PLpro protease. Structural proteins S, E, M and N are encoded by their respective genes, interspaced with accessory proteins which structural proteins indicated (Hardenbrook and Zang, 2021).

The reason for this is yet to be discovered. Although respiratory tract viruses are not spread through blood contact, there are cases of SARS-CoV-2 RNA been detected in blood samples which suggest that SARS-CoV-2 have the potential to be transmitted through blood transfusion or contact with blood-related materials such as needles. It is important to note that in spite of this mentioned facts; there is no report of transmission of SARS-CoV-2 via blood yet (Mclntosh, 2022).

Inter-human transmission is the dominant route for SARS-CoV-2 infection. Transmission occurs through close contact to infected individuals by inhaling particles from the sneeze and cough of infected people. An individual can get infected indirectly as well by contact with SARS-CoV-2 contaminated surfaces (Mclntosh, 2022).

Studies carried out on nine months pregnant women suggests that there is no evidence of pregnant women that got infected at the late stage of pregnancy transmitting SARS-CoV-2 to the child through intrauterine vertical transmission (Chen *et al.*, 2020).

However other experiments showed that there is a possibility of SARS-CoV-2 infected pregnant women transmitting the virus to the child (Ben and Guo, 2021).

ACE2 expression in reproductive organs facilitates SARS-CoV-2 to infect pregnant women and become a carrier (Jing *et al.*, 2020).

There are high genetic similarities of SARS-CoV-2 and the coronavirus from Pangolin in Malaysia (Xiao *et al.*, 2020). CoVs which are isolated from pangolins are the most related to *SARS-CoV-2* (Zhang *et al.*, 2020). This suggests that pangolins could be intermediate host of *SARS-CoV-2* (Wang *et al.*, 2020).

Intermediate hosts could transmit SARS-CoV-2 to susceptible individuals which lead to the appearance of diseases in humans (Ye *et al.*, 2020; Zhang *et al.*, 2020) SARS-CoV-2 can be transmitted between animals as well (Wang *et al.*, 2020).

Covid-19 patient can develop symptoms at early stages of infection (7 to 10 days), and can transmit it at this early stage, due to the high viral RNA load in the respiratory tract. This concept is confirmed by a study done in China. The onset of symptoms among 77 transmission pairs was 8.5 days, it was estimated that infectiousness peaked between 2 days before and 1 day after symptom onset, and declined within 7 days. Another study that was done in Taiwan evaluated over 2500 close contacts of patients with COVID-19. All 22 secondary cases had their first exposure to the index case within 6 days of symptoms onset. There was no infection evidence in the 850 contacts whose exposure was after this interval. SARS-CoV-2 symptoms can also depend on the levels of viral RNA in samples. Experiment demonstrates that high level of viral RNA in a sample leads to the detection of a viral infection which is done through a reverse transcriptase polymerase chain reaction (RT-PCR)(McIntosh, 2022). SARS-CoV-2 transmission route is shown in figure 3.

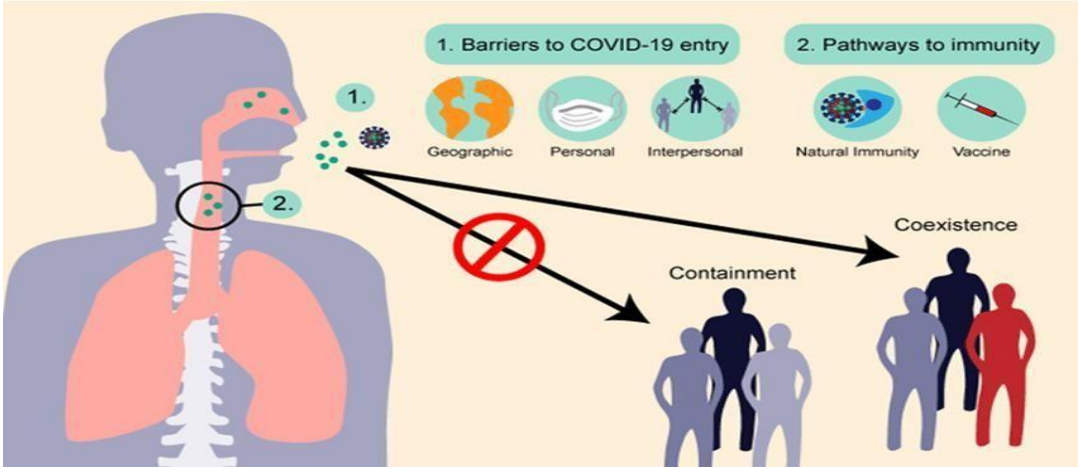

Figure 3: Viral transmission and outcome of COVID-19 infection (Chen *et al.*, 2020).

2.3.3. SARS-CoV-2 structural proteins and functions

A. SARS-CoV-2 spike glycoprotein

The *SARS-CoV-2* spike glycoprotein is found on the surface of the outer membrane of the virus and is 600 kDa trimer. SARS-CoV-2 spike protein is one of the largest fusion proteins. There is a 1300 amino acids precursor protein encoded by the spike gene of SARS-CoV-2. This protein is activated by the enzyme protease cleaving the S1 and S2 subunits into a N-terminal (S1) and C-terminal subunits. The formation of spike protein on the surface of the virion is a result of a heterodimer structure that was formed from the S1 and S2 subunits which were then oligomerized into a timer (Hardenbrook *et al.*, 2022).

The spike glycoprotein (S) is crucial for the viral uptake in coronaviruses (Li *et al.*, 2003).. The S1 have two domains, the N-terminal domain (NTD) and a receptor-binding domain (RBD). S1 helps to bind the virus to the host receptor cells. Unlike the S1 subunit, the S2 contains more domains and is more complex. It contains a fusion peptide (FP), heptad repeat 1 (HR1), central helix (CH), connector domain (CD), repeat 2 (HP2), transmembrane domain (TD), and a cytoplasmic tail (CT). The S2 subunit plays the role of fusion between virus membranes and the host cells. There is also a cleavage site found in between the subunits 1 and 2, which is known as S1/S2 protease cleavage site. This site is cut by the host protease enzyme to activate the proteins that are needed to fuse the virus's membrane to the host cells, via irreversible conformational changes (figure 5) (Wang *et al.*, 2020).

The spike protein also has two forms of structure as well, the open and closed conformation. The open conformation makes it easy for the virus to enter the host cells by allowing the fusion of host cells and viral membranes (Figure 4) (Walls *et al.*, 2020).

B. Receptor-binding domain (RBD)

The receptor binding domain (RBD) of SARS-CoV-2 play a role of recognizing and binding to specific receptors on the host cell which is the angiotensin converting enzyme 2 (ACE 2) (Wang *et al.*, 2020). Because of its role, the RBD is a target for antiviral antibodies (Letko *et al.*, 2020). The receptor-binding domain of *SARS-CoV-2* contains a core and an external subdomain's structure. The core is conserved, and it contains 5 beta strands that are in an antiparallel form in terms of their arrangement and a disulfide bond that is found in between2 beta strands (Wang Q *et al.*, 2020). While the external

Subdomain is primarily dominated by the loop that is stabilized by the disulfide bond (Wang Q *et al.*, 2020).

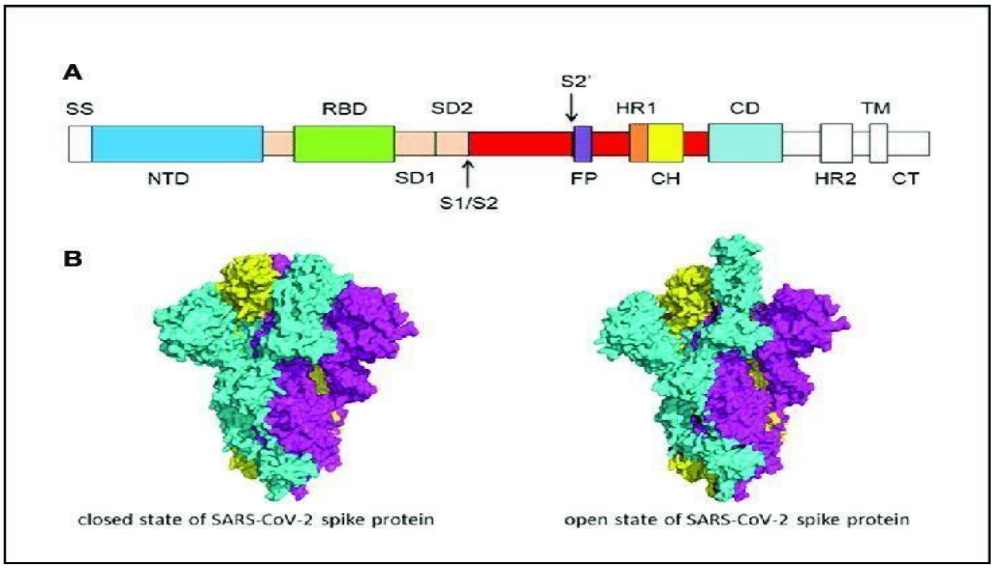

Figure 4: (A) Schematic structure of SARS-CoV-2 primary structure.
(B) Cryo-EM structure of SARS-CoV-2 spike protein (Wang et al., 2020)

The core of SARS-CoV-2 is made up of five beta sheets which are arranged in antiparallel manner and connected by loops and short helices. The receptor binding motif which contains loops and alpha helices and short 5 beta and 6 beta strands is found in between and beta7 strands. Most binding sites for SARS-CoV-2 and AEC-2 are found on the receptor binding motif (Wang *et al.*, 2020)

C. The Nucleocapsid (N) Protein

Among the different viral proteins, the N protein is the most abundant in *SARS-CoV-2*. Therefore, the N protein can be detected in COVID-19 patients samples during the early stage of infection. The N protein attaches itself to the RNA of the virus and forms a ribonucleoprotein core which plays a vital role in the entrance of *SARS-CoV-2* into the host cell (Satarker, 2020).

D. SARS-CoV-2 Envelope Protein

The envelope mediates viral budding, as a result of this, deletion of E protein is a potential way in viral pathogenicity reduction or removal, making it an effective method to produce anti-viral drugs and vaccine for SARS-CoV-2 (Hardenbrook *et al.*, 2021).

The envelope (E) protein is a small membrane protein that has 76 to 109 amino acids and 8-12 kDa in size. It has the N-terminal domain, a hydrophobic domain and a chain at the C-terminal domain. In their genomic structural sequence, the N-terminal covers the position 1 amino acid to position 9 amino acids, the hydrophobic domain starts from position 10 to the 37th amino acid position while the C-terminal domain is at the 37th to 76position of amino acid. These structures aid in virulence of the virus (Satarker, 2020).

E. The SARS-CoV-2 Membrane Protein

Like the N protein, the M protein is also abundant in Coronaviruses. The M protein is in two different forms found in the N-terminal ectodomain and others in the C-terminal end domain that does not have the same conformational structure. The M protein has about 220-260 amino acids and a short N-terminal domain (Satarker, 2020).

2.3.4 Ecology of SARS-CoV-2 and target cells

Depending on the cells that *SARS-CoV-2* infects, COVID-19 has three clinical stages (Mason, 2020). SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) (Trypsteen *et al.*, 2020).

To initiate infection, SARS-CoV-2 binds to the epithelium cells in the host nasal cavity followed by viral replication. The virus starts propagating at this stage. Host innate immune response is still low or limited at this stage. Usually, COVID-19 patients have no symptoms at this stage. Within few days after infection, SARS-CoV-2 propagates and migrates downward the host respiratory tract (lower respiratory tract) triggering a serious innate immune response, that induces clinically manifestations. In most COVID-19 patients, the virus does not proceed to phase three but about 20% of patients get to phase three (Mason, 2020).

At this stage, the virus migrates to the lungs and to the alveolar type II cells, making the patient difficult to breathe. In the alveolar type II cells, the virus propagates and infects more cells in that unit and releases large numbers of viral particles, which lead to the death of the host cell. Normally after apoptosis takes place, the innate immune response is highly triggered, and regeneration of the epithelium's cells occurs from type I cells. Elderly patients are mostly at risk during this stage three of the virus due to their compromised immune response. Since they have lower chance of their epithelium cells to be repaired and regenerated (Mason, 2020).

A systematic study was done in order to have a better understanding of different sites of infection of the virus in the human body and different symptoms and different organs expressing the angiotensin converting enzyme receptor figure 6 (Trypseen *et al.*,2020).
The damages done to the alveoli type II cells by SARS-CoV-2 are like that of the influenza virus and SARS-CoV-1 (Bridges, 2022). About 25% of COVID-19 patients often suffer from post-viral infection complications. The type I and type II alveoli cells are necessary for the regeneration of damaged alveoli epithelium in the post infection stage. When lung damage occurs, alveoli type II cells play the role of mature stem cells and then differentiate into alveoli type I cells and play a role in regeneration. *SARS-CoV-2* can infect both type I and II alveoli cells (Guptu, 2021). COVID-19 patients also suffer from vascular disorder because another organ or site that SARS CoV-2 target is the endothelium. Histopthalological studies have shown the effect of the virus on endothelium cells, which undergoes apoptosis, pyroptosis, and lymphocytic inflammation in the lungs (Brosnahan, 2020).

Pathologic studies on lungs of severe SARS-CoV-2 patients showed the presence of marked diffuse alveolar damage. They also reported that SARS-CoV-2 antigens and RNA are present in the alveolar epithelium and alveolar macrophages (CD 68+Cells) (Ding et *al.*, 2004 ; Franks *et al.*, 2003 ; Gu *et al.*, 2005 ; He *et al.*, 2006 ; Hwang *et al.*, 2005 ; Nicholls *et al.*, 2006 ; Shein *et al.*, 2005 ; To *et al.*, 2004 ; Ye *et al.*, 2007). These studies showed that alveolar epithelium cells are important in SARS-CoV-2 infection, which diffuse alveolar damage and respiratory failure (Mossel, 2007).

2.3.5 The human respiratory system

The nasal airway epithelium is the site where the virus replication and infection start. The primary target cells of SARS-CoV-2 are the ciliated cells. Before the virus propagates to other sites and organs, treatments are needed to prevent further spreading of the virus to the alveolar cells where the host is at high risk. There are two enzymes responsible for SARS-CoV-2 entry to the host cell. The angiotensin converting enzyme 2 (ACE2) receptor, which plays a role of recognizing and binding the virus to the host cell surface, and a transmembrane serine protease 2 (TMPRSS2) which helps to cleave the Spike protein to facilitate the viral entry (Bridges, 2021).

Naturally, the human airways epithelium has a defense role against pathogens by preventing infections and damaged tissues by secreting mucus and balancing airflow. Studies have shown how SARS-CoV-2 spike protein initially binds to the ACE2 receptor

and then the TMPSS2 will cut the S protein that facilitate the replication of

the virus in the infection site after an individual encounters the virus by inhaling it. Having an accurate understanding of the expression of the receptor ACE2 and the TMPSS2 protease can help in developing antiviral drugs by preventing the entry of the virus into the host cell. Inflammatory mediators such as CXCL10 (C-X-C motif chemokine 10) and interferon can be expressed by infected epithelial cells. Through results and observational studies, the use of drugs that can inhibit ACE2 expression is recommended for COVID-19 patients (Brosnahan, 2020) (Figure 7)

ACE2 expression is stimulated or activation by IL-1 beta and IFN-beta, and it is inhibited by IL-13 (Bridges, 2021).

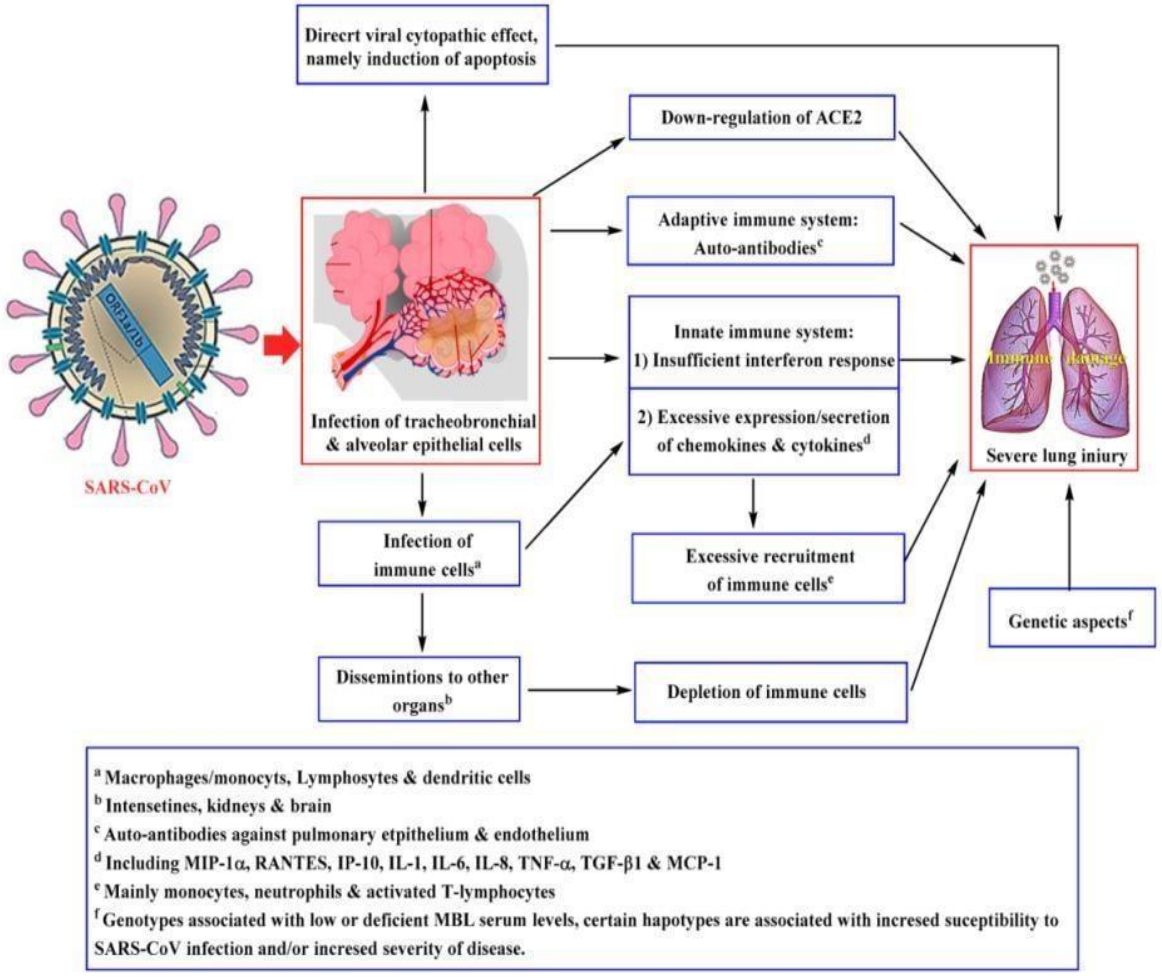

Figure 5: Important mechanism contributing to SARS-CoV-2 pathogenesis.

2.4 Human immune response to SARS-CoV-2 entry

After the individual encounters SARS-CoV-2 which gains access into the upper respiratory tract through or via the nasal epithelium, tonsils and adenoids nasopharynx-associated lymphoid tissue (NALT), where it leads to the inducing of mucosal immunity. The first type of host immunity that is activated is the innate immune. The innate immunity acts first via patterns recognizing receptors (PRRs) to pathogen-associated molecular patterns (PAMPs). Antiviral innate membrane (TLR7, TLR8 and TLR9) will then act directly on strong antiviral type I interferon response. The type I interferon act as a bridge or a link between innate immune response and activation of adaptive immunity response as well. Response of the host innate immunity limits the viral entry into the host cell, genome replication, proteins translation, assemblages of virions and identification of already infected cells (Primorac *et al.*, 2022).

The next response and activated cells against SARS-CoV-2 are the major histocompatibility complex (MHC) class I and class II and direct natural killer (NK) cells. Class II molecules of the major histocompatibility complex (MHC-II) are presented by on the antigen-presenting cell membranes such as the macrophages, monocytes, dendritic cells and B cells. These molecules (MHC-II) are necessary for activating, proliferation and differentiating B cells, and as well as CD 4+T cells. The expression of MHC-II by cells can be induced by interferon gamma (IFN-gamma) and be modulated by other factors as well. Example of these factors includes interleukin-4 (IL-4), interluekin-10 (IL-10), interferon alpha/beta (IFN-alpha/beta) tumor necrosis factor-alpha (TNF-alpha) and glucocorticoids. MHC I on the other hand, is expressed on all type of cells that have nucleus and is used to recognize antigens for CD 8+T cells (Primorac *et al.*, 2022).

About 5-20% of circulating lymphocytes and 15% of the total amount of blood found in the human body is made of the natural killer cells. The role of these natural killer cells is to detect infected cells caused by pathogens without interfering with the class I major histocompatibility complex (MHC I). Thus, making them less effective of disadvantaged when an intracellular pathogen invades CD 8+ cells by interfering with MHC I molecule expression (Primorac *et al.*, 2022).

2.4.1 Host T-Cell immune response against SARS-CoV-2

Definition: T cells are also known as T lymphocyte are leucocytes, a type of white blood cells that defense and protect the body from pathogens.

To control COVID-19 and eliminate SARS-CoV-2 infection, the host adaptive immunity is essential and needed. Studies have shown how T cells can mediate disease control in SARS-CoV-1 and MERS-CoV as well. There have been observations of declined or decreased inflammatory and clinical symptoms in SARS-CoV-1 infection when high levels of antibodies were indicated (Moss, 2022). After the release and availability of the genomic sequence of SARS-CoV-2, T cells immune response against SARS-CoV-2 was identified. According to research done by Peng and colleagues (2021) with the use of ELIS spot technology (fig.6), they observed that the amount or level of T cells response was related to the severity of SARS-CoV-2 patients (Moss, 2022).

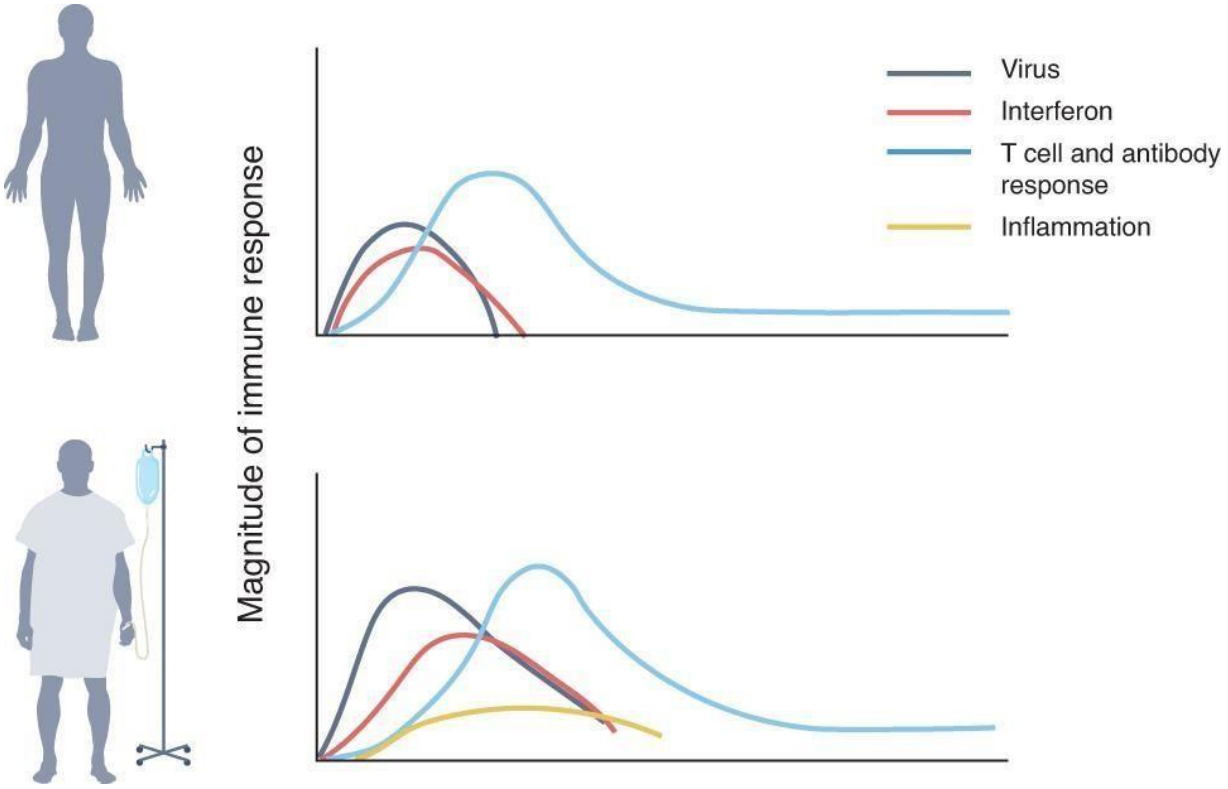

Figure 6: Representation of immune correlates in relation to clinical severity of during SARS-CoV-2 primary infection.

Effective clinical control of primary SARS-CoV-2 infection is associated with early and robust interferon and adaptive immune responses, which effectively control viral load. A delayed, inadequate and prolonged interferon response seems to be associated with slowed and elevated, cellular activation, with early inflammation and a poor clinical outcome (Moss, 2022).

The level of patient serum immunoglobulin and memory B cells are related with the mortality of COVID-19 patients and disease severity. The levels of B cells, Naive B cells, switched memory cells, and serum IgA, IgG, IgGI, and IgGII in severe COVID-19 patients and even deceased individuals were very low as compared to recovered patients (Primorac *et al.*, 2022).

The levels of SARS-CoV-2 neutralizing antibodies were observed to have declined during the early stages in patient recovery in both symptomatic and asymptomatic COVID-19 patients for most individuals who were infected previously. That suggests the effectiveness of neutralizing humoral response which is induced by SARS-CoV-2 vaccine may be relatively limited as well. Although healthy vaccinated individuals have shown higher levels of neutralizing antibodies titers than patients who have been recovered fromCOVID-19. Thus examining the levels of neutralizing antibodies titer or humoral response measure is one of the methods of understanding the severity of COVID-19 (Primorac, 2022).

The mucosal immunity is activated when SARS-CoV-2 enters via the nasal epithelium into the upper respiratory tract. Unlike neutralizing antibodies titers, the less measure or examine immunity is the musocal immunity. Inside the mucosal subepitheluim and other related glands, the mucosal plasma produces IgA which is then transported into the secretions and will be released as SIgA (Secretory IgA) as shown in figure 7SIgA can also inhibit viral attachment to the host cell surface and as well as epithelial cells invasion, enabling agglutination and removal of mucus (Primorac *et al.*, 2022).

Specific T cells that response to SARS-CoV-2 spike protein are dominated by CD 4+ and may help in antibody generation with follicular helper T cells that is correlated with humoral immunity in memory phase (Primorac *et al.*, 2022).

Memory T cells are maintained within the first 12 months following clearance of infection by CD 4+T cell population and CD 8+ T cell populations which comprise 0.5% and 0.2% of the repertoire and target at least 19 epitopes and 17 epitopes respectively. T1/2 half-life (Moss, 2022)

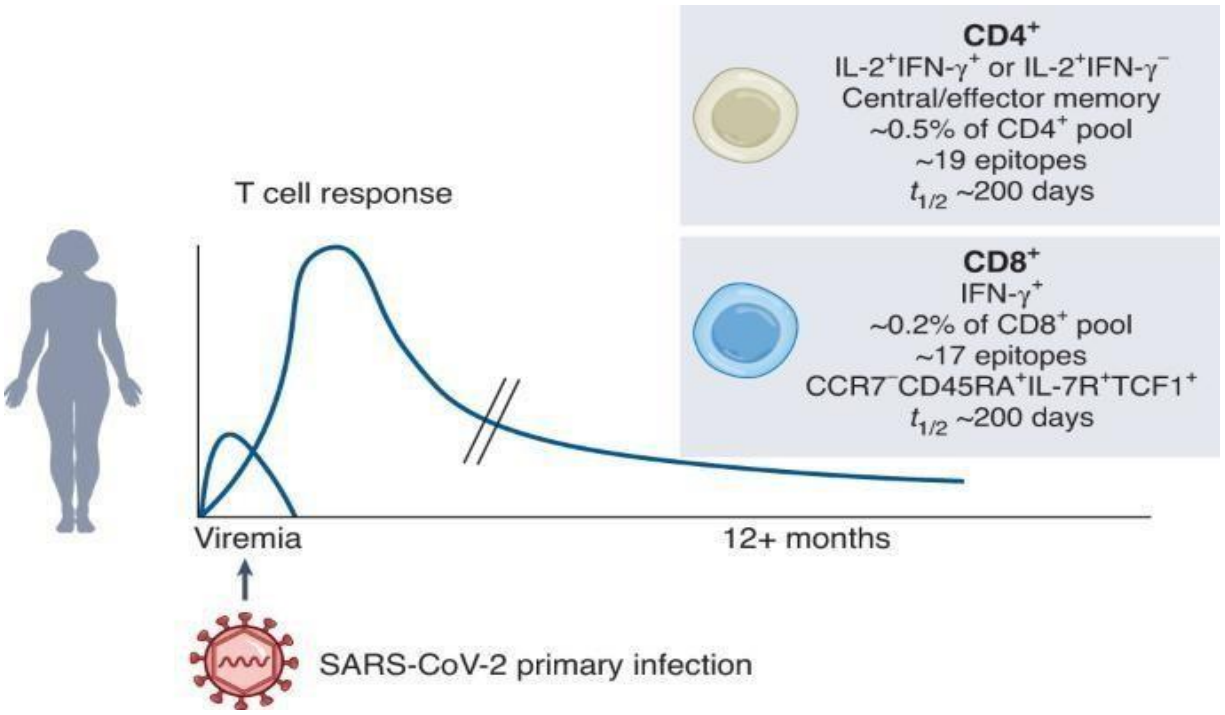

Figure 7: Profile of T cell immune memory to SARS-CoV-2 following the clearance of Primary infection (Primorac et al., 2022).

CHAPTER II

Chapter II: SARS-CoV-2 vaccine development and different vaccines production Strategies

1. History of vaccination

The need to develop a vaccine against viruses had been practicing for a long period of time. Several human viruses have emerged and some of these viruses subdued over time without vaccine. Vaccination started in the 1700s by Edwards Jenner during his studies to prevent smallpox that killed about 300-500 million people before the virus was eradicated in 1979 (Plotkin *et al.,* 2011). Several and new strategies are developed in vaccine making based on genetic engineering, structural and biological systems. The aim of these new strategies is to provide vaccines which can induce a protective immune response in the host (Plotkin, 2011). Figure 8 shows a history timeline of vaccination.

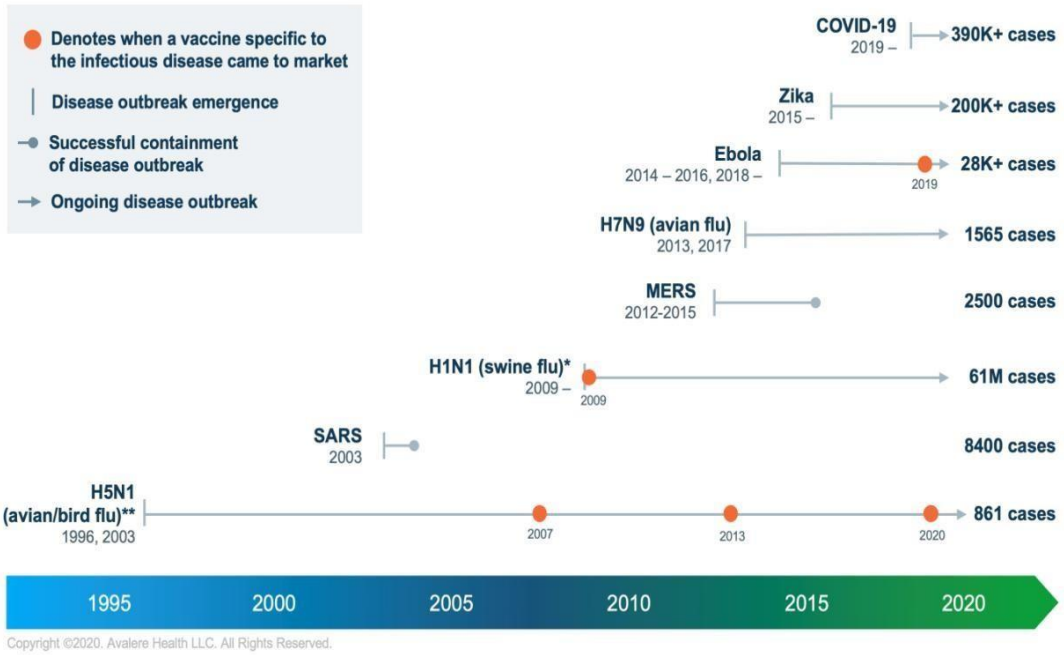

Figure 8: History timeline of different emerged human virus vaccine Development (Johns Hopkins COVID-19 global cases)

2. Vaccination against human viruses

Vaccination against human viruses is very important to the public health to stop viral spread and infections. Vaccination has played a major role in public health protection. Especially, since national programs for immunizations were initially established and coordinated in the 1960s till date. Vaccines are designed to activate the host immune system to response antigens(Pollard and Bijker, 2021). A representation of vaccination and activation of the host immune system is shown in figure 9.

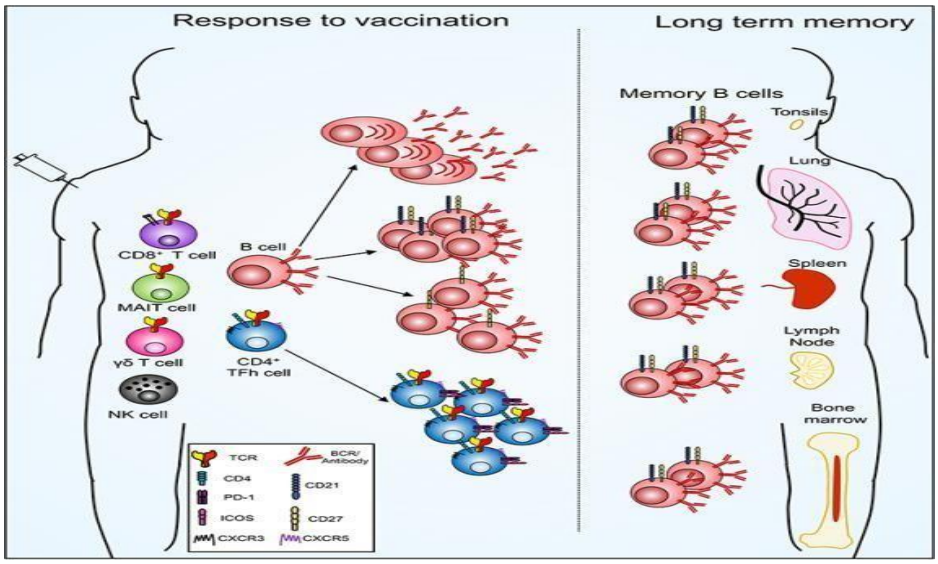

Figure 9: Representation of vaccination processes and the activation of the body Immunity system (Kedzierska et al., 2021).

The right side of the diagram shows, how the host memory B cells are Activated for long-term memory against the pathogen

3. SARS-CoV-2 evolution and variants

SARS-CoV-2 continues to spread globally because of different emerging variants. Viruses can change their genome as it spreads overtime which is known as mutation. A variant occurs when there is a new mutation in the genetic code of a virus or a pathogen. When viruses

undergo any mutation in any part of the genome, it can lead to change their initial characters (Lauring *et al.,* 2021).

A. Viral mutation

Mutation: Is a change that takes place in the genome of a virus that can sometimes lead to a change in the character of that virus (Zhou B. *et al.,* 2022).

Variant: Is a viral genome that has gone through a mutation and has one or more changes (Zhou *et al.,* 2022).

Patients were reported to have cases of reinfection with *SARS-CoV-2*. This is a result of the virus mutation. Some of the mutations do not affect the primary structure of the virus. The variants increase the transmission of the virus, increase the human ACE2 receptor binding affinity, viral replication, escape host immune response. Although, gene mutation can occur in any part of the genome, the new character of the virus also depends on the location of mutation. *SARS-CoV-2* gene mutation in the ORF8 shows high rate of the viral transmission and immune evasion potential because its gene also has a role in RNA polymerase complex and controlling host major histocompatibility complex class 1 (Hirabara *et al.,* 2022). Most of these mutations occur in the spike protein of *SARS-CoV-2*. Other mutations sites were found in the receptor binding domain (Peng, 2021). Considering the mentioned above facts, there isno prevalence of the current available *SARS-CoV-2* vaccines being effectiveness against new strains of the virus (Dong *etal.,* 2021).

B. SARS-CoV-2 spike protein mutation

Mutations in the receptor binding domain (RBD) can affect antibodies neutralizing activity in *SARS-CoV-2* because the epitope has about 90% antibodies neutralizing activity on previous COVID-19 infected patients (Dong *et al.,* 2021).

4. SARS-CoV-2 variant classifications

WHO has classified different *SARS-CoV-2* variants into variants under investigation (VUI), variants of interest (VOI) and variants of concern (VOC). VOI and VOC have become the

main concern for the health community. These variants have the potential of changing the nature of the virus. Studies have shown that VOC increase the chances of harmful changes in the COVID-19 epidemiology. These VOC also enables the virus to become more ineffective to existing vaccines, alteration of existing clinical manifestations, social control majors and diagnostics. While, on the other hand, the VOI cause changes in the viral genes which make the virus to have more characters of high transmission rate (Peng *et al.*, 2021).

A. Lineage B.1.1.7 (alpha Variant)

The lineage B.1.1.7 (alpha variant) was identified in the United Kingdom in 2020. Having more significant ability in increasing the cases of COVID-19. It was considered as a variant of concern on 18 December 2020. This variant has about 22 mutations characters, which include 13 non- synonymous mutations, 6 synonymous mutations and 3 deletions. The mutations in this variant include the N501Y mutation of the spike protein. The N501Y mutation of the SARS- CoV-2 spike protein increases the affinity of receptor binding domain with the host ACE2. Another mutation that was found in the alpha variant is the P681H mutation which has a role of facilitating the viral entrance into the host cell. This variant also has a higher transmission range of 40-70% as compared to other SARS-CoV-2 variants. In December 2020,this variant caused one quarter of total COVID-19 cases globally (Hirabara *et al.*, 2022; Parra- Lucares *et al.*,2022).

B. Lineage B.1.351 (βeta Variant)

This lineage has one of the mutants in spike protein of the alpha variant lineage, the N501Y and various other structural and non-structural mutations including K417N, E48K in the receptor binding domain of Spike protein of *SARS-CoV-2*. The K417N makes *SARS-CoV-2* to hide from immune response andrender*SARS-CoV-2* vaccine to be less efficacy. The E48K in the other hand increase viral binding to the ACE2 receptor and decreases responses to host antibodies which are triggered during COVID-19 infection (Parra-Lucares *et al.*, 2022; Hairabara *et al.*, 2022).

C. Lineage P.1 (Gamma Variant)

The lineage P.1 variant was identified in January 2021 in Tokyo, Japan. It is responsible of 42% COVID-19 cases. This variant has mutants such as the N501Y K417T, and E484K in the spike protein. This lineage cause SARS-CoV-2 to be more infectious to host cell receptor binding affinity and high transmission rate, resistance to the host immune response (Parra- Lucares *et al.*, 2022)

D. Lineage B.1.617 (Delta Variant)

The delta variant of SARS-CoV-2 was identified in 2021 from samples that were collected from COVID-19 patients in 2020 by the Indian Ministry of Health, having characters of rendering the virus to escape immune response (Parra-Lucares *et al.*, 2022).

The lineage of B.1.617 is divided into three sub lineages. The B.1.617.1, B.1.617.2, B.1617.3. B1.617.2. These mutants have higher transmission range compared to the initial strains of SARS-CoV-2 and have high risk of hospitalization. Although individuals that have been vaccinated against the original strain (B.1.617 variant) of the virus can still be infected with different emerging variants of the virus and transmit it. Mutation of L452R, T478K and that of E484Q occurs in the spike protein receptor binding domain and P681R in the cleavage site that is in position of S1 and S2 in SARS-CoV-2 delta variant (Hairabara *et al.*, 2022).

E. LineageB.1.1.529/BA.1 (Omicron Variant)

The omicron SARS-CoV-2 variant was reported and identified in South Africa. However, it was observed in other countries before. It was considered as a variant of concern by WHO because of its epidemiologically range of COVID-19 caused in South Africa. This variant caused about 280 to 800 daily new cases in the following weeks after the new SARS-CoV-2 variant was reported. It is yet to be discovered if the high rate of the virus is as a result of this variant. As previously detected SARS-CoV-2 variants, the Omicron variant had mutants such as the N501Y, E484A-E484K, T478K; P681H-P681R (Parraa-Lucares *et al.*, 2022;Hairabara *et al.*, 2022).

The Omicron variant of SARS-CoV-2 has several mutations including more than 30 mutations that occurs in the spike protein, but there are no studies showing if this variant cause COVID-19 severity and death rate. Epidemiological data suggests that this variant can infect individuals 3 to 6 times more than the variant B.1617.2 making this variant the variant more transmissible than other variants (Parra-Lucares *et al.*, 2022).

5. Types of SARS-CoV-2 available vaccines

5.1 DNA Vaccine

Vaccines that are made from viral DNA are promising to combat viruses because DNA vaccines are easy to produce in large scale. Its flexible cold chain for storage and rapid is an advantage. There are several vaccine platforms that have adapted DNA-based vaccines technology to fight SARS-CoV-2. Although this technology has some challenges and needs more development, it is one of the adapted technologies in vaccines development (Kyriakidis*et al.*, 2021).

DNA-based vaccines are made up of a viral gene in a DNA plasmid and delivered into the host cell which expresses itself and triggers the host immune response. One of the advantages of this type of vaccine is that the viral gene can be produced in a plasmid in large scale by using bacteria. The vector used in this type of vaccine for SARS-CoV-2 contains SARS-CoV-2 gene, eukaryoticpromoter that can express the viral antigen which codes for the Spike protein (Li *et al.*, 2016).

One of the challenges about DNA vaccines is that they cannot induce enough immunopathogenesis because they lack the ability to self-amplify *in vivo*. As a result of this, there are several strategies that are used in preclinical model trials such as using plasmid vectors, antigen promoter for its expression, electroporation, adjuvants, prime boost regimens to increases immune response (Lei Li and Petrovsky, 2016).

SARS-CoV-2 proteins including the M, N and S protein can induce different levels of host immunity response. Among the different structural proteins of SARS-CoV-2, the Spike (S) protein has been observed to induce a sufficient level of host immunity that is protective. These

Vaccines that are based on SARS-CoV-2 spike protein can fight this virus without causing post-immunization problems. Even though, past studies showed that the chances of getting a vaccine plasmid into the host chromosome can be difficult (Li *et al.*, 2020).

Yang at al. (2021) used mice injected with vectors generated with the viral DNA with its full length of the spike protein, the spike protein that does not have the cytoplasmic domain, and spike protein that does not have cytoplasmic and transmembrane domain as well. By immunizing the mice with these three vaccines, all the three results showed neutralizing antibodies induction, T-cell immune response and protective response in the mice. Based on the results from this experiment, SARS-CoV-2 full-length spike protein DNA-based vaccines reached phase 1 clinical trial. The experiment showed that this type of vaccine was effective, protective and tolerated by SARS-CoV-2 patients and induced neutralizing antibodies against the virus with T-cell response in healthy adults (Li *et al.*, 2020). Because of these results, SARS-CoV-2 DNA vaccine candidate (INO-4800) was proceeded to phase I/II clinical trial (NCT04447781 and NTC04336410) considering the fact that it uses the same technology and is designed as GLS-5300 MERS-CoV DNA-based vaccine (Li *et al.*, 2020). From all these studies, DNA-based vaccines whether it encodes the full length of the spike protein or the S1, have been proven to be effective and protective to fight SARS-CoV-2.

5.1.1 The pcDNA3.1 plasmid as example of DNA vectors used in DNAvaccine production

A. Plasmid components and *in vivo* amplification

As described by Xingyun and collaborators in 2022, the spike protein sequence of the tested vaccine candidate was retrieved from available SARS-CoV-2 whole genome sequences that were published in NCBI. Optimized DNA sequence was synthesized firstly and then digested with BamH1 and Xhol. The next step was to clone in PCDAN3.1 vector (figure 11) with cytomegalovirus immediate-early promoter and bovine growth hormone polyadenylation signal. For amplification of targeted gene, the plasmid was transformed into DH5 α competent cells by using heat shock-ice bath method for 30 min, heat in 42°C water bath for 90 seconds, and another ice bath for 5 minutes. The bacteria were incubated on a solid Luria-

Bertani (LB) medium that contained ampicillin and then incubated at 37°C for 16 h. The aim of this step was to identify and select ampicillin resistant bacterial cells. The single colony was detected using PCR, and the correct bacterial colony was transferred into 2.5L conical flask, then placed inside 37°C incubator, and shaken at 250 r/min for 12 h.

After this cultivation was done, the liquid medium was centrifuged, and the bacterial pellets were kept in a -80°C refrigerator. Extraction of the plasmid was performed on the bacterial pellet using the protocols of the plasmid DNA extraction kit (Vazyme, Nanjing, China) (Xingyun et al., 2022).

B. *In Vitro* validation of DNA vaccine expression

For trials investigation, the DNA vaccine was injected into mice on day 0, 14 and 28.pcDNA.31-spike was diluted with PBS to 25, 100, 200, 400μg/mL, each group of animals comprised four mice (Figure 11). The used volume of intramuscular injection was 100μL for each mouse, and the same was applied to the control group. Next was the detection of plasma IgG and antibody titers via ELISA technique (Xingyun *et al.*, 2022).

5.2 RNA vaccines

RNA-based vaccines are made up with the viral antigenic encoding information that is translated into the viral antigen. Both non-self-replicating mRNA and self-replicating mRNA are used (Krammer, 2020). Non-self-replicating mRNA vaccines encodes the viral target antigen and contains 5′ and 3′ untranslated regions. While the self-replicating mRNA encodes not only the target viral antigen but encodes as well a replicas complex which enables intracellular amplification of the vaccine RNA and enhances protein expression (Thomas and Knut, 2017; Norbert et al., 2018a). Unlike the self-replicating RNA, the use of non-self-replicating mRNA requires higher doses. RNA vaccines are not delivered alone, but requires lipid nanoparticles (LNPs) (Krammer, 2020).

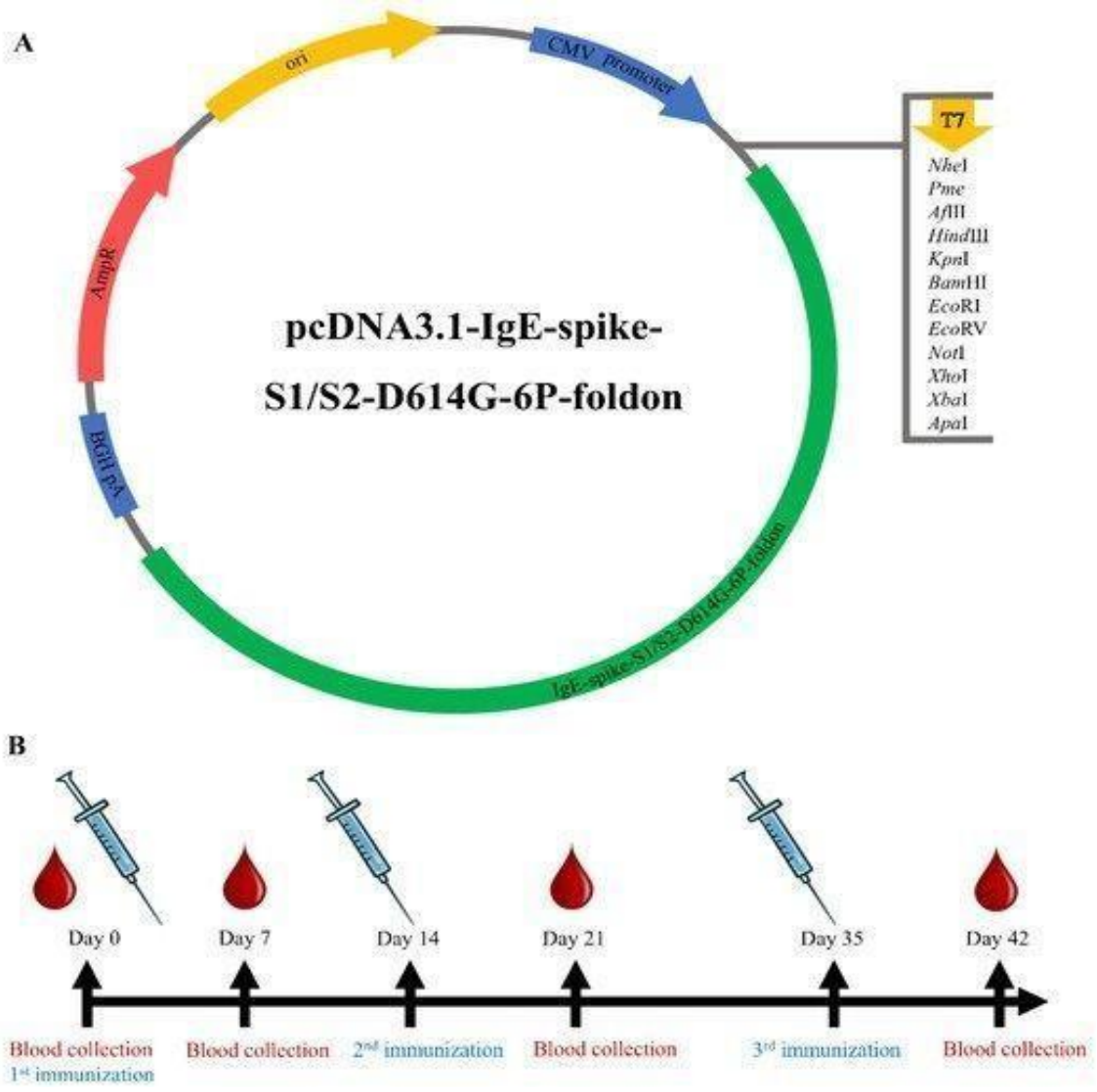

Figure 10: Structure of pcDNA3.1 plasmid.

This plasmid was used in SARS-CoV-2 DNA vaccine production

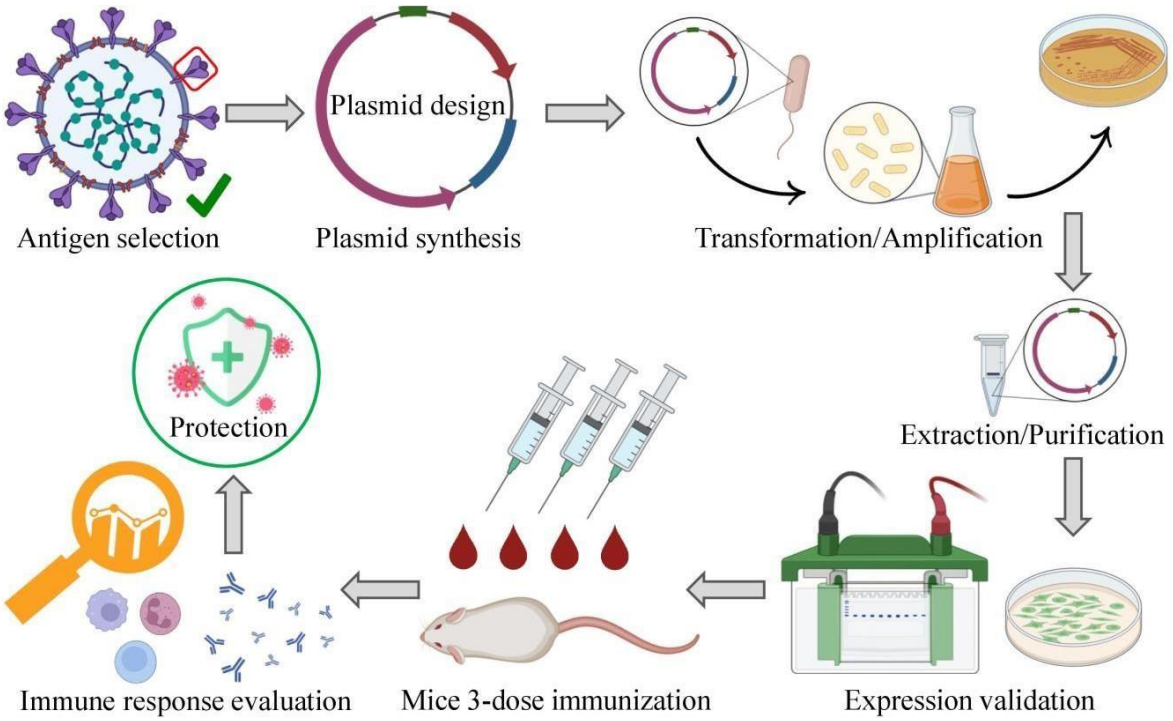

Figure 11: Diagram of main steps of production of DNA vaccine (Liji, 2020).

Schematic diagram showing how SARS-CoV-2 DNA vaccine candidate

Is produced using a plasmid to clone and expressed target genes.

5.2.2 Methods in SARS-CoV-2 RNA vaccine candidate

A. Cloning Vector

Amplification of target DNA was performed from COVID-19 patient sample. The DNA was sequenced, confirmed, and modified to achieve desired structure as such it harbors a suitable 5′ UTR, ORF to express the S protein with G614 with double proline mutation (K986P and V9879) with IgE-secretory signal sequence, a special 3′ UTR was constructed with modified alpha and beta globin in tandem, and a 130 residue-long poly-A tail.

The vaccine was produced by performing in the presence with 3′-0-Me-m7G (5′) ppp (5′) G RNA Cap Structure Analog and S-adenosylmethionine using MEGA script™ T7 transcription

kit (ThermoFisher, USA) and Ribonucleotide solution Set (NEB, USA). In Vitro transcription mRNA synthesis reaction was done. The reaction was run at 37°C for 2 hours, and DNase treatment was performed for 15 minutes at 37°C. Following a dephosphorylation step, Using Antarctic Phosphatase (NEB, USA). The mRNA was purified with the use of phenol:chloroform:isoamyl alcohol, with MEGA clear™ Transcription Clean-Up Kit (Kakon Nag et al., 2021).

For identification, the purified mRNA was formulated and treated with RNAse samples, and then analyzed using size elusion chromatography. Dilution using sodium acetate buffer was performed after the elusion chromatography. After different steps of formulation was performed, the vaccine was tested for immunogens (Kakon Nag et al., 2021).

B. Challenges in RNA Vaccines

RNA vaccines are unstable. The RNA can lose its structure and requires very low temperatures to remain stable. The cold chain of RNA vaccines is between -70°C and -20°C which renders their scale-large production, distribution and long-term storage difficult. Another inconvenient of these types of vaccines is that vaccines that are administered via injection have lower amount of inducing sufficient mucosal immunity in the individual (Krammer, 2020).

5.3 Inactivated Vaccines

For these types of vaccines instead of using a live virus like that of attenuated vaccines, the virus is first inactivated using chemical products in the laboratory. They are safer compared to that of live virus vaccines because it does not have the risk of the virus been reactivated. In thecase of SARS-CoV-2 that can mutate and escape host immunity, inactivating the whole virus can be a solution even for patients that their response to treatment is long due of certain health conditions which sometimes give the virus advantage to mutate. The processes of inactivating live virus have some risks. Vaccinated individuals' health complications with inactivated vaccines against MERS-CoV and SARS-CoV have been observed. The virus can be structurally damaged during viral inactivation. This can make the virus not inducing or stimulating thehost immune response (Li *et al.*, 2020).

SARS-CoV-2 inactivated vaccines are achieved by growing SARS-CoV-2 in Vero cells and inactivated using chemicals. So far there are some SARS-CoV-2 inactivated based vaccines such as CoronaVac or PiCoVac by Sinovac Biotech in China (Krammer, 2020).

5.3.1 Procedures of SARS-CoV-2 inactivated vaccines

The following steps are shown from the publication of Saad in 2021 to explain the laboratory scale production of inactivated SARS-Cov-2 virus.

A. Sample collection

Nasopharyngeal and oropharyngeal swabs were collected from positive tested patients using RT-PCR in 5 mL viral transport media (Saad, 2021).

B. Virus Isolation

Epithelial cells from African green monkey kidney (Vero 6E cells) were used and maintained in Dulcecco modified Eagle medium (DMEM) that is supplemented with 10% heat inactivated fetal bovine serum and 100 mM L-glutamine. The bovine serum was used to maintain the medium and incubated at 37° cover night (Saad *et al.,* 2021; Wang *et al.,* 2005; Pavel *et al.,* 2021).

The Vero 6E cells were infected with P4 viral stock in multiple infection of 0.001 at temperature between 33°C and 37°C. The viral stock used was obtained from CN2 SARS-CoV-2 strain that was purified and passed once in the Vero cells to generate P1 stock. After which, four other passages were performed to generate P4 and P5 stock (Gao *et al.,* 2020; Wang *et al.,* 2020).

C. Viral identification

The cells were observed and examined twice daily during incubation for cytopathic effects caused by the inoculated virus, followed by removal of supernatants for viral load by rRT-PCR, cytopatic effect, gene detection and electronic microscope was used to confirm viral replication (Saad *et al.,* 2021).

D. Real-Time PCR detection

The viral RNA was extracted using RNA extraction kit (Qaigan, CA). The extracted RNA was checked for purity and concentration with NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). One step rRT-PCR was performed using TaqPath COVID-19 CE-IVD RT-PCR Combo Kit following the manufacturer's instructions. The reaction was incubated in rRT-PCR ABI 7500 (Thermo Fisher Scientific, USA) at 50°C for 15 minutes for reverse transcriptase step, 95°C for 10 minutes followed by 45 cycles of 95°C for 15 seconds and 60°C for 30 seconds. Three primers/probes were used to target the viral ORF1ab region, N and Spike. Primers/probes specific for bacteriophage MS2 were used as positive control. The cycle threshold value below 33 was considered as positive. The results are valid when two of the three targeted genes and the MS2 showed positive results (Saad *et al.,* 2021).

E. Whole Genome Sequencing

Quantification of extracted RNA was performed using Qubit RNA High Sentivity Kit (Invitrogen, USA). Libraries were prepared using Ion AmpliSeq SARS-CoV-2 Kit (Thermo Scientific, USA) following the manufacturer's instructions. Clonal amplification of the libraries was done using Ion-PI-Hi-Q sequencing 200 Kit (Thermo Scientific, USA). Purified libraries were quantified and qualified by Agilent Bioanalyzer and Qubit 4 Fluoremeter (Thermo Scientific, USA). The libraries were then sequenced using the Ion proton NGS platform (Thermo Scientific, USA). Virus sequences assembly conducted with the Ion tolerantpackage (v.5.12) followed by mapping of genome with tmap program (v.512) against SARS-CoV-2 whole genome sequence submitted (Saleh *et al.*, 2021).

F. Viral inactivation

Harvested viruses were inactivated with formaldehyde (1:4000 v/v) for 48 hours to lyse the viral membrane, followed by purification via chromatography and concentrated. A secondviral inactivation was done using β-propiolactone 1:2000 (v/v) in order to destroy the viral genome followed by another purification step and protocol (Huang *et al.,* 2021). Viral inactivation can be done with BBP as well (Cerutti *et al.*, 2021; Pavel *et al.*, 2021), and some cases, heat inactivation or UV inactivation can be used (Loveday *et al.*, 2020).

Viral inactivation was confirmed by inoculation of β-propriolactone-treated samples in Vero E6 cells. The inactivated virus was filtered using a 0.45um filter (Millipore-sigma) following polyethylene glycerol precipitation. Precipitated viral supernatant was treated with 20U/mL of benzonase overnight at 2-8°C to digest the host cell DNA. Purification was done using column chromatography and then followed by tangential flow filtration system, which is a method used in biological field for protein separation (Millipore cogent). After a sterile filtration is done, the whole inactivated viral vaccine was formulated (Pavel *et al.*, 2021).

G. Transmission Electronic Microscope

Infected Vero cells were then removed from the flask, pelleted and rinsed with 0.1M phosphate buffer saline (PBS) (Sigma Aldrich Germany) (Pavel *et al.*, 2021). Collected cell supernatants of Vero cells monolayers which has been inoculated were fixed with 2% formaldehyde in PBS for 1h followed by ultracentrifugation (1h, 25,000 rpm), and then a carbon coated grind stained with 2% phosphor-tungestic acid was used to load samples for 30 seconds for examination. Bar: 100 nm (Saleh *et al.*, 2021).

H. Virus Titration

Infectious dose (TCID50) per ml was determined in Vero cell monolayers on 24 and 94-well cell plates. Serial dilutions was done to test CPE on cells of virus samples, incubated at 37°C for 4 days. TCID50 assay was conducted according to Ksiazek *et al.* The infectious titer was calculated using an in-house method adopted by Spearman and Karber and expressed in TCID50 units (Saad *et al.*, 2021).

I. Vaccine Preparation and validation of inactivation

To prepare the vaccine, the virus was propagated in Vero cells with a dilution of 1:100 (v/v) of SARS-CoV-2 in serum-free medium, and then the cells were incubated at 37°C for 72h. On day three post infection, visible supernatants with CPE were collected for cell harvesting. Determination of infectious titer was done using 50% cell culture infectious dose. Cell harvest clarification and vaccine purification were conducted at low centrifugation speed of 1000 rpm. SARS-CoV-2 was then chemically inactivated with 37% diluted formaldehyde at 37°C for 24

h. The final formulation was liquid formulation, containing 55 µg or 100 µg total proteins with aluminum hydroxide (Alhydrogel® CRODA health care Corp) as adjuvant (0.45 mg/mL) per

0.5 ml. In order to verifier the inactivated virus, inoculations of Vero monolayers was done in 75cm2 flasks with 10 ml of the inactivated virus, and cultured at 36 ± 1°C for 4 days. The results showed no CPE for three passages, and also quantitative PCR (Q-PCR) was performed at many time points duringpassage confirmed that there is no amplified virus genomes.

J. Vaccine Safety Test in mice

Two groups (8 mice in each group) of 3–4-week-old Swine albino mice were provided by VSVRI. Safety assessment was done according to Animal and Plant Health Inspection Service (USDA) and Institutional Animal care and Use Committee (IACUC). The group one was injected intraperitonneally with 0.5 ml of the inactivated vaccine that contains 5 mg of Al (OH)3 which represented about 25-8 human doses that recommended (0.2-0.8mg). The group 2 was injected with 0.5 ml phosphate buffered saline (PBS) as a control group, and then clinical manifestations and other effects were observed (Saad *et al.*, 2021).

H. Inactivated SARS-CoV-2 vaccine trial in human

The trial step is divided into four. Phase1, the vaccine is administered in volunteers without any health complications, Phase 2, the vaccine is given to small number of individuals in orderto know the safety of the vaccine. In phase 3, in the phase the vaccine disease preventing efficacy is determined the phase 4 is conducted after the vaccine safety have been confirmed and approval guarantee (Marcelle et al., 2020)

This vaccine can be inactivated with formaldehyde or aluminum hydroxide. In the clinical trial carried out on humans, Data analysis of randomly selected 2 groups of individuals were used for placebo-controlled trials. Phase I trial was conducted on 96 randomly selected adults. After different doses were given to selected individuals over days, the inactivated SARS-CoV-2 vaccine showed low level of immunogenicity and low rate of adverse (Shengli et al., 2020).

Primary immunopathogical neutralizing antibodies response in day 14 of postvaccination which measured by a 50% plaque reduction neutralizing test against SARS COV 2. From this, inactivated SARS-CoV-2 vaccine was observed to have a low rate of immunopathogenic effects.

K. Immunogenicity analysis of vaccine

Three groups of 6-week-old swine albino mice (n=10) were immunized with the trial vaccine on day 0 at two doses. The first and second group immunized with 0.1 ml and 0.2 ml, regardlessvia intramuscular route, and re-vaccinated with the same doses on day 7. The group 3 (Controlgroup) was injected with physiological saline. Blood was collected from the animal's tail veins, and antibody neutralizing assay was performed to analyze the vaccine's immunogenicity (Saad*et al.*, 2021).

5.4 Live Attenuated Vaccines

Live attenuated vaccines are made from live virus that has lost its genomic pathogenic characters. They mimic the precursor of the virus having almost the full immunogenic structures of the virus. Since these vaccines are based on the original viral antigens, it can replicate and cause host immune response just like the natural response during an infection caused by the original virus.. Viral attenuation is obtained by exposing the virus to unfavorable growth conditions. This can be achieved by growing the virus in non-human cells by changing its growth temperature or deleting genes that give the virus its pathogenic features (Krammer, 2020).

These types of vaccines can induce mucosal immunity that protects the upper respiratory tract which is a primary route of entrance by SARS-CoV-2 through intranasal administration to the

individual. Since the virus replicates itself inside the individual, it stimulates the host immune response and triggers the host immune system to target the whole structure of the virus including structural and non-structural proteins and activate cellular and antibodies response (Krammer,2020). Since the virus is weakened, it can become pathogenic again in the cases of

patients with health issues and have a weak immune system just like the immunocompromised individuals. The virus has opportunity to reverse back to the virulence (Li*et al.*, 2020).

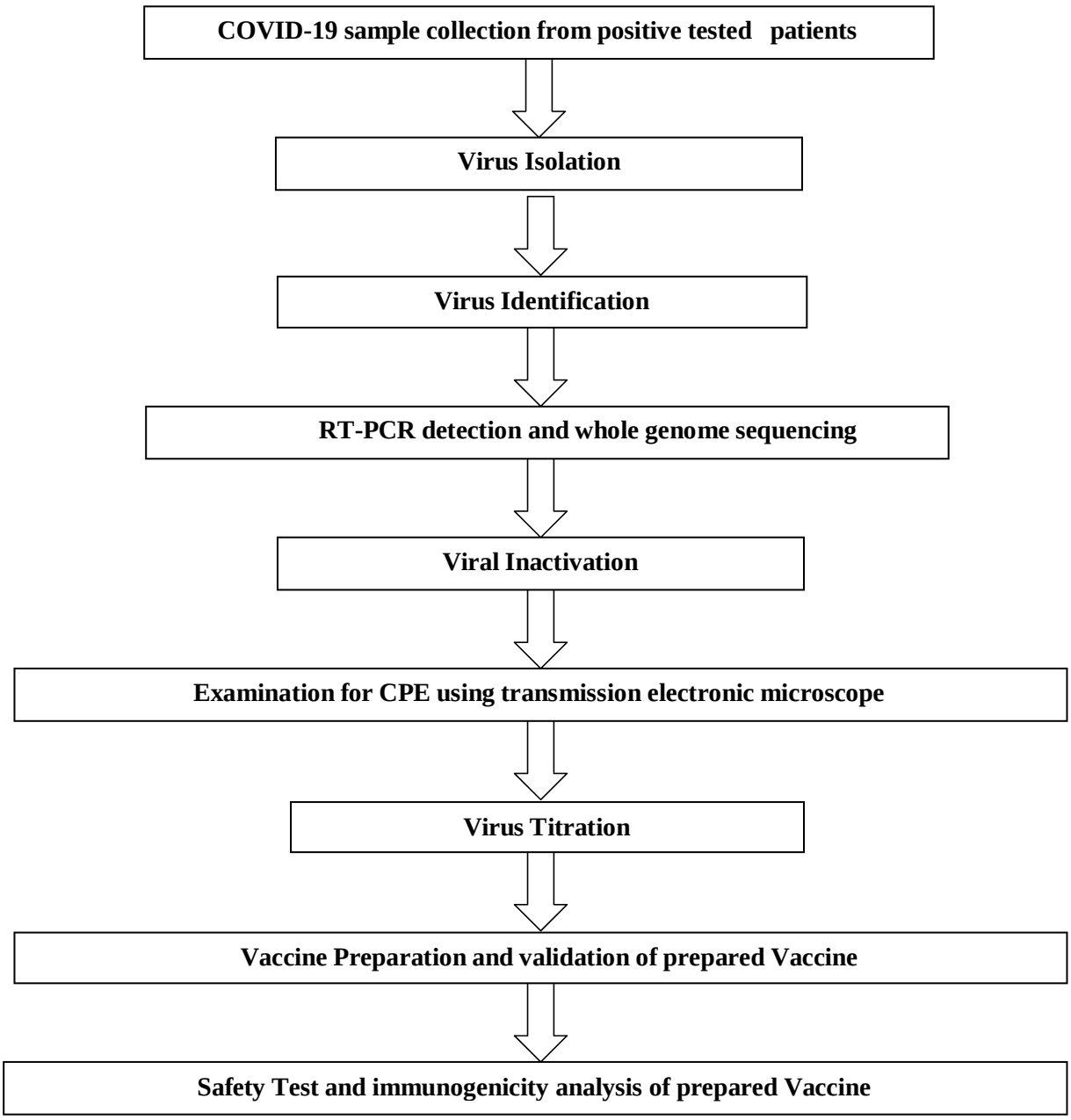

Figure 12: Schematic steps of SARS-CoV-2 Inactivated Vaccine Procedures.

5.4.1 Strategies of live attenuated vaccines

To develop live attenuated vaccine, different structural proteins of the virus can be weakened to render the viral genome in a pathogenic form but still can replicate so as to stimulate the host immunity. The envelope (E) protein is one of the targets for SARS-CoV-2 vaccines development. Studies show that the envelope protein does not just have a structural function but also it can activate inflammasome and cause inflammatory in the lungs. In regard to this ability or role in lung activating lung inflammation, deleting the E protein of SARS-CoV-2 is targeted in developing vaccines (Li *et al.,* 2020).

Another important structure to target in developing coronaviruses vaccines is the Nsp16. The non-structural protein 16 (Nsp16) encodes the enzyme ribose 2′-O-methytransferase which has a role in the extremity of 5′ capping of the viral RNA. The methylation plays a role protecting the virus from disintegration and to avoid activation of type 1 interferon-dependent innate immune response by the RNA of the virus. Nsp16 mutant vaccines have been reported to fight SARS-CoV and MERS-CoV with protective effects (Li *etal.,* 2020). A study done by Graham *et al.,* showed that the deletion of exonuclease induces SARS-CoV-1 pathogenicity or virulence in aged mice, younger ones and even immunocompromised mice.

5.5 Viral Vector Vaccine

These types of SARS-CoV-2 vaccines are different from the traditional methods. Instead of using the whole virus or viral antigens into the host body, the genetic code for the viral antigen is inserted in a vector which delivers the viral antigenic code into the host cell. These types of vaccines trigger strong host immunity. They do not require any use of adjuvants or need of booster doses. Table 4 shows some other platforms of currently available SARS-CoV-2vaccines with new developed technologies. Different vaccines have specific doses and the time interval per dose. The required doses of some SARS-CoV-2 vaccines are indicated in the same table.

Table 3: Examples of the available SARS-CoV-2 vaccines (Li et el., 2020)

platforms	Vaccine types	Developer	doses	Clinical trial phases
DNA vaccine	NCoV	Zydus cadia	3 (1D)	III
Viral vector vaccine	Covid-19 vaccine AsraZeneca	AstraZeneca + University	1-2 (M)	IV
RNA vaccine	mRNA-1273	Modera and national institute of allergy and infectious disease	2 (1M)	IV
Recombinant vaccine SARS-CoV-2	EpicVacCorona	Federal budgetary research institution state research center of virology and biotechnology	2 (1M)	III
Protein Subunit vaccine	UB-612	Vixxinity	2 (1M)	III

D= day interval. M=Month

6. Post vaccination challenges

The vaccines are not 100% safe or effective for everyone because each person is different.

The host immune system has a role of defense against intruding antigens by activating specific types of cells which targets and clear off the antigen. The host immune system is capable to have a memory of these antigens and leads to rapid effective response when exposed to the same antigen. However, these responses can sometimes be inappropriate such as auto-immune responses (Jean et al., 2018).

Some of the challenges after the administration is the allergic reactions, which are more common with RNA-based vaccines, like Pfizer-BioNTech and Moderna. These vaccines have been observed to have immunopathological effects (Salman and Gajendra, 2020). Therefore, itis recommended for individuals to not get second dose when cases like this are observed.

SARS-COV-2 inactivated vaccines are highly effective. Although, there are causal illness because of these types of vaccines. Neutralizing antibodies that were induced in clinical trials after second dose of DNA-based vaccine dropped after months. The specific memory B and T cells were still detected, which forms the basis for rapid recall response (Yihao andHaipeng, 2022). Consequently, the need to use other molecules to fight Covid-19
are highly recommended for health promotion.

6.1 SARS-CoV-2 inhibitors and neutralizing antibodies.

A. Convalescent Plasma

Emergency use of convalescent plasma was approved by the FDA as of 2020. The idea is to use convalescent plasma from recovered COVID-19 patients to treat infected individuals. Convalescent plasma from recovered COVID-19 patients contains primarily antibodies which neutralize SARS-CoV-2 antigen and can potentially inhibit the entry of the viral into the host cell. This method has been understood to be effective and limits the duration of pathogens in the host cell. Even though this has been the case, the use of convalescent plasma against SARS-CoV-2 has shown some limitations and inconstancy (Chitsike and Duerksen-Hughes, 2021).

B. Anti-SARS-CoV-2 Monoclonal Antibodies

Monoclonal antibodies are synthetic or fabricated antibodies. These antibodies are produced from convalescent plasma, transgenic mice, and B cell isolation. Challenges like

heterogenecity is very limited with the administration of these antibodies. Therefore, it’s an effective way to fight SARS-CoV-2 (Chitsike and Duerksen-Hughes, 2021).

The principle of this technique is to fabricate antibodies in the laboratory and used them to block the entry of SARS-CoV-2 and as well as prevent viral spreading in the host cells. An important point to note is that unlike vaccines which trigger the host immunity against viral antigens which needs a period of time, synthetic antibodies give the host the required antibodies to fight the virus without triggering the host immunity. But a downside of these

types of antibodies is that they can cause mild allergic reactions in the individual (U.S. Department of health and human services 2020).

C. Anti-SARS-CoV-2 Nanobodies

Nanobodies are small size, single domain antibodies that are produced from immunized llamas, phages display and camels (Chitsike Penelope Duerksen,2021). As a result of their small size, they can access protein cavities which conventional antibodies cannot access. Studies have shown how nanobodies neutralize circulating variants and prevent immune escape variants by binding to various non-overlapping epitopes. They bind to conserved epitopes which cannot be accessed by other antibodies (Hong *et al.*, 2022). Figure 13 describe neutralizing nanobodies of SARS-CoV-2.

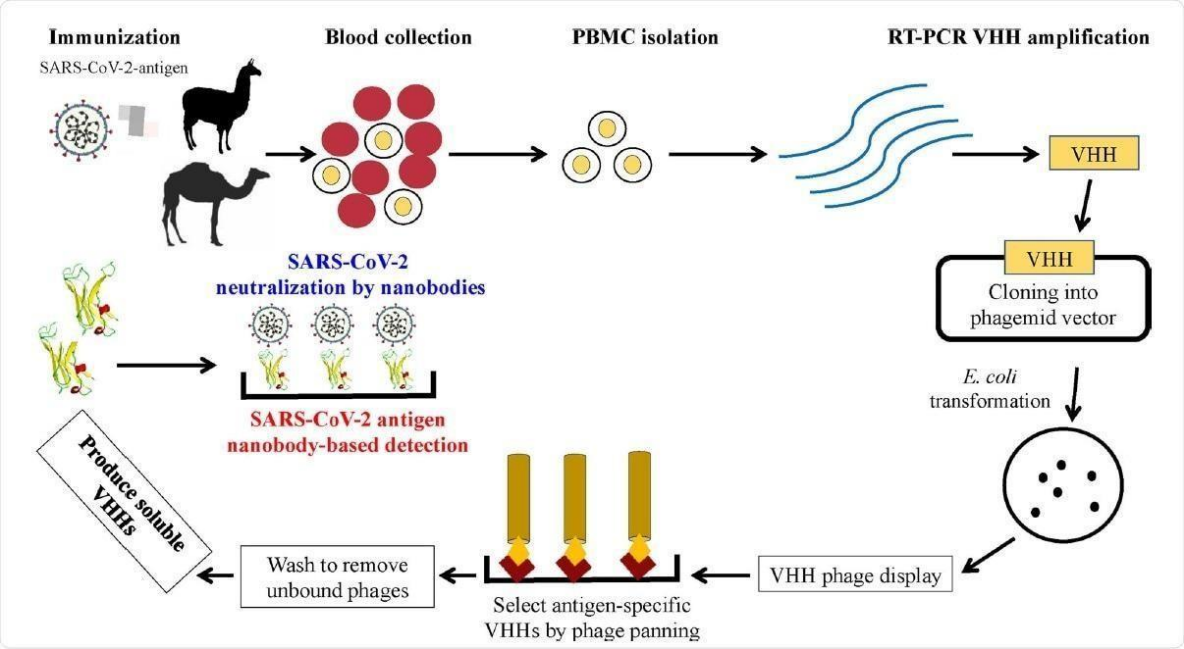

Figure 13: Schematic representation of heavy-chain variable domains (VHHs) generation process and their potential applications as therapeutic agents and as well as diagnostic tools for COVID-19 pathology (Liji, 2022).

D. Protease enzyme inhibitors

SARS-CoV-2 attachment to the host cell and membrane inside the cells in order to release its genetic material is mediated by the viral subunits S1 and S2. The host protease enzymes (Furins, cathepsins and surface serine protease) plays a major role in the function of these subunits. The particular type of host protease that participates in the viral activities depends on the route on which the virus uses to enter the host cell. SARS-CoV-2 can enter its host cell either by direct contact with the surface membrane or through endocytic uptake. When the viral entry is through surface membrane contact, the host protease in use will be furin. The S1/S2 site of SARS-CoV-2 will be cloven by the enzyme furin proprotein convertase. Exposing or revealing the S2 site to the surface trypsin-like serine protease such as TMPRSS2.The S2 site is instantly upstream of the fusion peptide and cleaves by TMPRSS2 exposing the hydrophobic peptide for insertion into the surface membrane (figure 14). On the other hand, if SARS-CoV-2 enters its host cell through endocytic pathway, cathepsins will participate the in the viral activities. Cathepsin L isoform have a major role in cleaving the S2 site in coronaviruses. The cathepsin L is lysosmal cysteine protease. Cathepsin L is pH-dependent and have an optimal pH of 3 to 6.5. These enzymes have a role of cleaving the viral spike and enabling it to fuse with the liposomal or autolysosomal membrane in order to release the viral genetic material into the host cytoplasm. A description of this mechanism is shown in figures 14 and 15 (Duerksen-Hughes, 2021).

E. Soluble Human AEC2

Another potential anti SARS-CoV-2 target is the human AEC2. SARS-CoV-2 uses the ACE2 as its receptor to enter the host cell. Thus, developing drugs that have chemicals to neutralize or block the binding of SARS-CoV-2 to this receptor is a potential method against SARS-CoV-2. A recombinant human ACE2 have shown to inhibit both SARS-CoV-1 and SARS-CoV-2 infections *in vitro*. Recombinant human ACE2 block the viral entry into the cell as well as regulate renin-angiotensin system in order to reduce the damages caused by the virus (Primorac *et al.*, 2022).

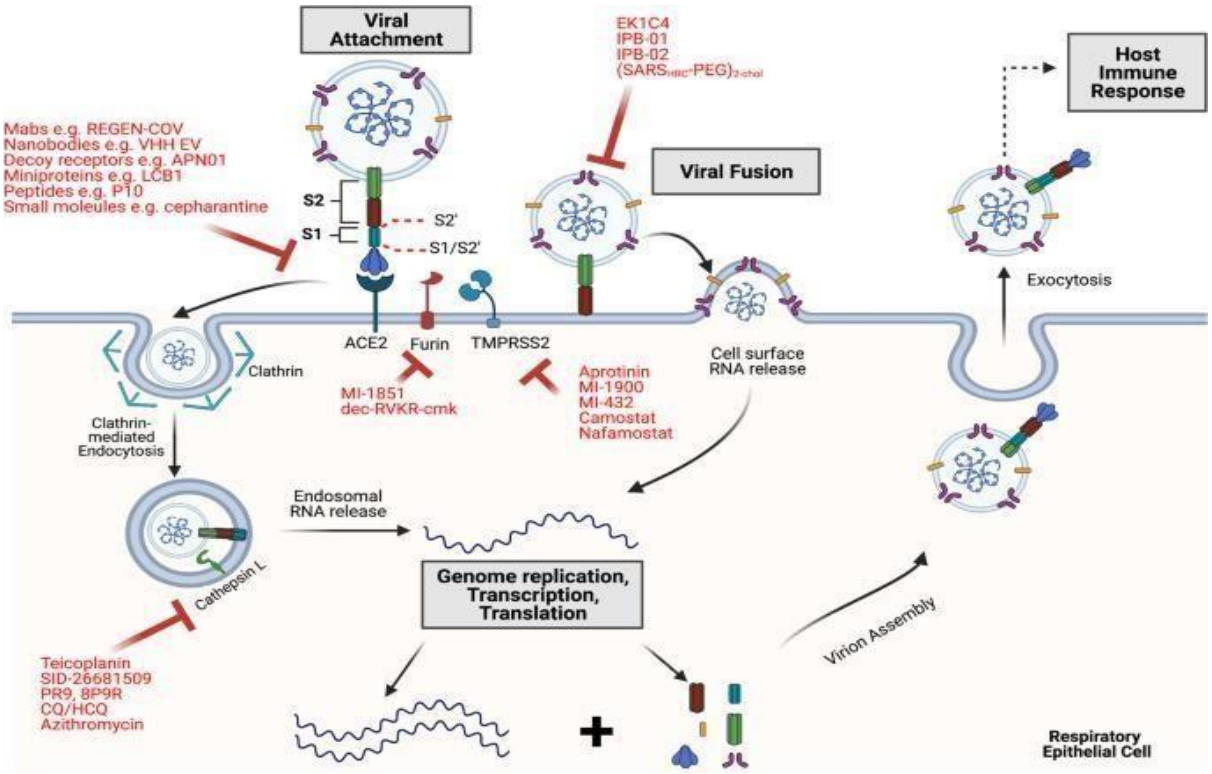

Figure 14: life cycle of SARS-CoV-2 and inhibitors of viral entry.

SARS-CoV-2 binds to ACE2 on the host cell surface membrane followed by spike activation by the furin and TMPRSS2. In the cytosol, the genomic RNA is translated from the ORF1a/b into polyproteins containing nonstructural proteins of the complex replicate machinery. The positive sense RNA is synthesized and becomes a template for positive sense genomic RNA and sub-genomic RNA from which structural proteins and accessory proteins are made. These proteins and the genomic RNA will be used to assemble new virions that will exit the host cell through exocytosis. The virions and death of infected cells will then induce host immune response (Lennox and Duerksen-Hughes., 2021).

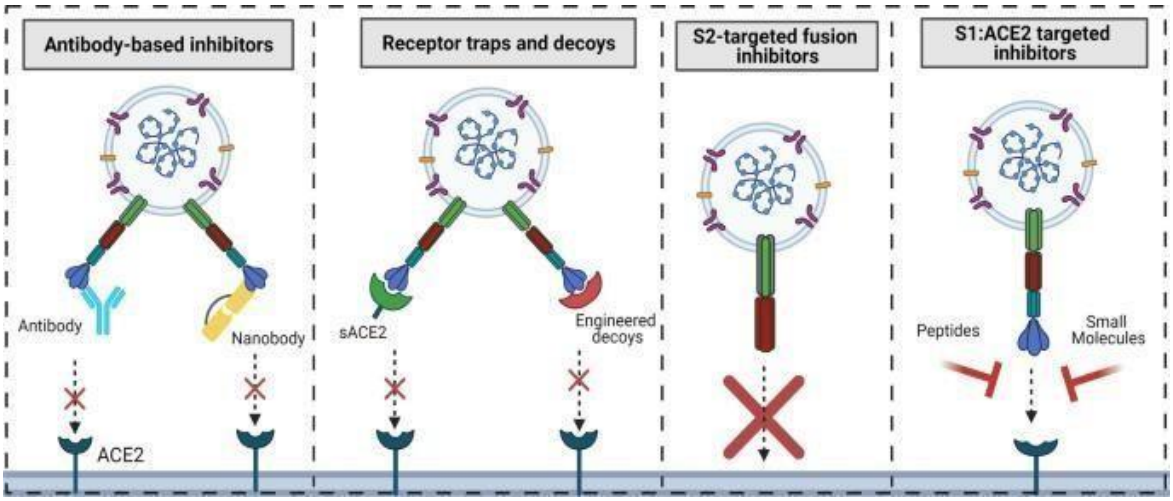

Figure 15: SARS-CoV-2 entry at the host surface membrane.

Four approaches are highlighted including antibody-based inhibitors that consists monoclonal antibodies and nanobodies. Receptor decoys consist of WT soluble ACE2 or versions ofACE2 that are engineered to have high affinity than WT ACE2. Various inhibitors based on HR2 of S2 have also been designed to prevent fusion of the S2 with cellular membrane. Peptides and small molecules that are designed to interfere with the S1 RBD and ACE2 interaction have also been made (Lenox and Duerksen-Hughes, 2021).

7. Post Vaccination T Cells Response for SARS-CoV-2

Several SARS-CoV-2 vaccines have been produced and have high levels of protection. These vaccines are in particular related to the disease severity and mortality in terms of their protection. Due to the level at which SARS-CoV-2 is highly contagious and the range of death, these vaccines efficacy is essential. And it is crucial that vaccines must have a defined T cell response. Spike specific cellular response is measured in most of the vaccine's registration. Usually within 11 days of the first vaccination, safe clinical effects and strong CD 8+T cell response are observed in the individual (Moss, 2022).

T cells response is needed in order to help generate and maintain high affinity antibodies, such in the case of BNT162b2. Dual Vaccination with BNT162b2b induces virus-specific

CD 4+T cell response in the individual. The response of CD 4+T exhibit or show T11 1 profile and can be detected by day eight after priming, peak soon after vaccine boost, and then fall

back to pre-boost levels after 4 months. Point to note, the induction levels of T cell response depends on the vaccine subtype (Moss, 2022).

The most important role of T cells to consider is its ability to provide a protective immunity against SARS-CoV-2. Antibodies response in humans during infections provides a safe and protective immunity against the initial stage of viral infections and also to induce viral specific neutralizing antibodies before vaccination. But there has been suggestion that cellular response may also have an important role in preventing initial productive infection (Moss, 2022).

After SARS-CoV-2 gains entry into the host cell and infection occurs, the host adaptive immunity is activated and clears off the virus. Antibodies neutralize the virus but there is a possibility for the virus becoming resistance to these antibodies as the virus is able to spread from one cell to another. Several other viruses have been observed using this mechanism. This suggests that there is a need for T cell immunity viral clearance (Moss, 2022).

The levels and intensity of viral load and effectiveness of the host innate immunity that is mediated by type I interferon are needed for clinical outcomes and subsequent adaptive response. Severe COVID-19 clinical outcomes are characterized by a decline in viral load, and prolong inflammation with high levels of interferon (IFN-alpha, TNF and IFN-Gamma) (Moss, 2022).

Conclusion and Perspectives

SARS-CoV-2 is one of the most highly pathogenic human coronaviruses which have emerged speedily causing worldwide global human, economic and social crisis. As of 6:25 p.m. CEST, 1 July 2022, there have been 545,226,550 confirmed cases of COVID-19, including 6,334,728 deaths, reported to WHO. As of 30 June 2022, a total of 11,986,040,938 vaccine doses have been administered.

The different available SARS-CoV-2 vaccines were manufactured after passing several phases, from exploratory stage to quality control stage, including clinical development and regulatory review and approval.

We discussed the conventional and new promising approaches that are used to manufacture the available vaccines. Life attenuated and inactivated virus-based vaccines are prepared using the whole virus structure after culturing in Vero cells, and then inactivated by chemical treatments. One example of these type of vaccines is Sinovac, produced in China and administered to the Algerian population. Another is the DNA plasmid-based vaccine, Ex. Sputnik made in Russia. This vaccine is generated using a specific plasmid vector that includes the gene of SARS-Cov-2 spike protein. mRNA based vaccines are a promising therapeutical tool in vaccine industry because mRNA is easily degraded by cellular processes.

Vaccination is the most effective way to stop the viral spread. The variability of SARS-CoV-2 is a challenge to the global public health till date, because available vaccines were designed to combat the first strain of SARS-Cov-2. Therefore, developing new methods are required to fight SARS-CoV-2 variants. SARS-CoV-2 vaccines must be able to induce appropriate protective immunity even for immunocompromised patientsto avoid post vaccination immunopathological effects. Consequently, the need to use other molecules to fight Covid-19 are highly recommended for health promotion.

Till now, almost one billion people in lower-income countries remain unvaccinated. Therefore, vaccines should be made available for free to less developed countries, while keeping all the preventive majors that have been implanted by governments and WHO.

References

Bahrami, A. and Ferns, G.A. (2020). Genetic and pathogenic characterization of SARS-CoV-2: a review. *Future Virology*, *15*(8), pp. 533-549.

Barbeau, D. J., Cartwright, H.N., Harmon, J. R., Spengler, J. R., Spiropoulou, C. F., Sidney, J., Sette, A. and McElroy, A.K. (2021). Identification and Characterization of Rift Valley Fever Virus-Specific T Cells Reveals a Dependence on CD 40/CD 40L Interactions for Prevention of Encephalitis. *Journal of virology*, *95*(23), pp. 1506-21.

Bian, L., Gao, F., Zhang, J., He, Q., Mao, Q., Xu, M. and Liang, Z. (2021). Effects of SARS-CoV-2 variants on vaccine efficacy and response strategies. *Expert review of vaccines*, *20*(4), pp. 365-373.

Bridges, J.P., Vladar, E. K., Huang, H. and Mason, R. J. (2022). Respiratory epithelial cell responses to SARS-CoV-2 in COVID-19. *Thorax*, *77*(2), pp. 203-209.

Bridges, J.P., Vladar, E. K., Huang, H. and Mason, R. J. (2022). Respiratory epithelial cell responses to SARS-CoV-2 in COVID-19. *Thorax*, *77*(2), pp. 203-209.

Brosnahan, S. B., Jonkman, A. H., Kugler, M. C., Munger, J.S. and Kaufman, D. A. (2020). COVID-19 and respiratory system disorders: current knowledge, future clinical and translational research questions. *Arteriosclerosis, thrombosis, and vascular biology*, *40* (11), pp.2586-2597.

Case, J. B., Rothlauf, P. W., Chen, R. E., Liu, Z., Zhao, H., Kim, A. S., ... and Whelan, S. P. (2020). Neutralizing antibody and soluble ACE2 inhibition of a replication-competent VSV-SARS-CoV-2 and a clinical isolate of SARS-CoV-2. *Cell host & microbe*, *28*(3), 475-485.

Chamings, A., Nelson, T.M., Vibin, J., Wille, M., Klaassen, M. and Alexandersen, S. (2018). Detection and characterisation of coronaviruses in migratory and non-migratory Australian wild birds. *Scientific reports*, *8*(1),

Cui, J., Li, F. and Shi, Z.L. (2019). Origin and evolution of pathogenic coronaviruses. *Nature reviews microbiology*, *17*(3), pp. 181-192.

Ding, Y., He, L.I., Zhang, Q., Huang, Z., Che, X., Hou, J., Wang, H., Shen, H., Qiu, L., Li, Z. and Geng, J. (2004). Organ distribution of severe acute respiratory syndrome (SARS) associated coronavirus (SARS- CoV) in SARS patients: implications for pathogenesis and virus transmission

pathways. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland*, *203* (2), pp. 622-630.

Dong, J., Zost, S.J., Greaney, A.J., Starr, T.N., Dingens, A.S., Chen, E.C., Chen, R.E., Case, J.B., Sutton, R.E., Gilchuk, P. and Rodriguez, J. (2021). Genetic and structural basis for recognition of SARS-CoV-2 spike protein by a two-antibody cocktail. *BioRxiv*.

Edwards, R. J., Mansouri, K., Stalls, V., Manne, K., Watts, B., Parks, R., Janowska, K., Gobeil, S., Kopp, M., Li, D. and Lu, X. (2021). Cold sensitivity of the SARS-CoV-2 spike ectodomain. *Nature structural & molecular biology*, *28*(2), pp. 128-131.

Fitzgerald, G. A., Komarov, A., Kaznadzey, A., Mazo, I. and Kireeva, M. L. (2021). Expression of SARS-CoV-2 surface glycoprotein fragments 319–640 in E. coli, and its refolding and purification. *Protein expression and purification*, *183*, p.105861.

Fong MW, Gao H, Wong JY, Xiao J, Shiu EYC, Ryu S, Cowling BJ. (2020). Nonpharmaceutical Measures for Pandemic Influenza in Nonhealthcare Settings-Social Distancing Measures. *Emerging Infectious Diseases*.; 26(5):976-984. doi: 10.3201/eid2605.190995.

Ganesh B, Rajakumar T, Malathi M, Manikandan N, Nagaraj J, Santhakumar A, Elangovan A, Malik YS. (2021). Epidemiology and pathobiology of SARS-CoV-2 (COVID-19) in comparison with SARS, MERS: An updated overview of current knowledge and future perspectives. *Clinical Epidemiology and Global Health*. doi: 10.1016/j.cegh.2020.100694

Ganesh, B., Rajakumar, T., Malathi, M., Manikandan, N., Nagaraj, J., Santhakumar, A., Elangovan, A. and Malik, Y.S. (2021). Epidemiology and pathobiology of SARS-CoV-2 (COVID-19) in comparison with SARS, MERS: An updated overview of current knowledge and future perspectives. *Clinical epidemiology and global health*, *10*, p.100694.

Gao, J., Zeng, L., Yao, L., Wang, Z., Yang, X., Shi, J., Hu, L., Liu, Q., Chen, C., Xia, T. and Qu, G., (2021). Inherited and acquired corona of coronavirus in the host: Inspiration from the biomolecular corona of nanoparticles. *Nano Today*, *39*, p. 101,161.

Gao, J., Zheng, P., Jia, Y., Chen, H., Mao, Y., Chen, S., Wang, Y., Fu, H. and Dai, J. (2020). Mental health problems and social media exposure during COVID-19 outbreak. *Plos one*, *15*(4), p. e0231924.

Gu, J., Gong, E., Zhang, B., Zheng, J., Gao, Z., Zhong, Y., Zou, W., Zhan, J., Wang, S., Xie, Z. and Zhuang, H. (2005). Multiple organ infection and the pathogenesis of SARS. *The Journal of experimental medicine*, *202* (3), pp. 415-424.

Hardenbrook, N.J. and Zhang, P. (2022). A structural view of the SARS-CoV-2 virus and its assembly. *Current Opinion in Virology*, *52*, pp. 123-134.

He, L., Ding, Y., Zhang, Q., Che, X., He, Y., Shen, H., Wang, H., Li, Z., Zhao, L., Geng, J. and Deng, Y. (2006). Expression of elevated levels of pro- inflammatory cytokines in SARS- CoV- infected ACE2+ cells in SARS patients: relation to the acute lung injury and pathogenesis of SARS. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland*, *210* (3), pp. 288-297.

Hirabara, Sandro M., et al. (2022). "SARS-COV-2 variants: differences and potential of immune evasion." *Frontiers in cellular and infection microbiology*:1401.

Hu, B., Guo, H., Zhou, P. and Shi, Z.L. (2021). Characteristics of SARS-CoV-2 and COVID-19. *Nature Reviews Microbiology*, *19*(3), pp. 141-154.

Hu, B., Guo, H., Zhou, P. and Shi, Z.L. (2021). Characteristics of SARS-CoV-2 and COVID-19. *Nature Reviews Microbiology*, *19*(3), pp. 141-154.

Hu, B., Guo, H., Zhou, P. and Shi, Z.L. (2021). Characteristics of SARS-CoV-2 and COVID-19. *Nature Reviews Microbiology*, *19*(3), pp. 141-154.

Hwang, D. M., Chamberlain, D. W., Poutanen, S. M., Low, D. E., Asa, S.L. and Butany, J. (2005). Pulmonary pathology of severe acute respiratory syndrome in Toronto. *Modern pathology*, *18*(1), pp. 1-10.

Krammer, F. (2020). SARS-CoV-2 vaccines in development. *Nature*, *586* (7830), pp. 516-527.

Kumar, A. and Nayar, K. R. (2021). COVID 19 and its mental health consequences. *Journal of Mental Health*, *30*(1), pp. 1-2.

Kumar, A., Singh, R., Kaur, J., Pandey, S., Sharma, V., Thakur, L., Sati, S., Mani, S., Asthana, S., Sharma, T. K. and Chaudhuri, S. (2021). Wuhan to the world: the COVID-19 pandemic. *Frontiers in cellular and infection microbiology*, 11, 596,201.

Kumar, B. and Pinky, S.D. (2021). Addressing economic and health challenges of COVID- 19 in Bangladesh: Preparation and response. *Journal of public affairs*, *21*(4), p. e2556.

Kumar, S., Kashyap, P., Chowdhury, S., Kumar, S., Panwar, A. and Kumar, A. (2021). Identification of phytochemicals as potential therapeutic agents that binds to Nsp15 protein target of coronavirus (SARS-CoV-2) that are capable of inhibiting virus replication. *Phytomedicine*, *85*, p.153317.

Kumar, V., Dhanjal, J. K., Kaul, S.C., Wadhwa, R. and Sundar, D. (2021). Withanone and caffeic acid phenethyl ester are predicted to interact with the main protease (Mpro) of SARS-CoV-2 and inhibit its activity. *Journal of Biomolecular Structure and Dynamics*, *39*(11), pp. 3842-3854.

Kyriakidis, N.C., López-Cortés, A., González, E. V., Grimaldos, A. B. and Prado, E. O. (2021). SARS - vaccines strategies: a comprehensive review of phase 3 candidates. *npj Vaccines*, *6*(1), pp. 1-17.

Lauring, A. S. and Hodcroft, E. B. (2021). Genetic variants of SARS-CoV-2—what do they mean? *Jama*, *325* (6), pp. 529-531.

Letko, M. and Munster, V. (2020). Functional assessment of cell entry and receptor usage for lineage B β-coronaviruses, including 2019-nCoV. *BioRxiv*.

Li, A., Xue, M., Attia, Z., Yu, J., Lu, M., Shan, C., Liang, X., Gao, T.Z., Shi, P.Y., Peeples, M.E. and Boyaka, P.N., 2020. Vesicular stomatitis virus and DNA vaccines expressing Zika virus nonstructural protein 1 induce substantial but not sterilizing protection against Zika virus infection. *Journal of virology*, *94*(17), pp. e00048-20.

Li, G., Chen, X. and Xu, A. (2003). Profile of specific antibodies to the SARS-associated coronavirus. *New England Journal of Medicine*, *349* (5), pp. 508-509.

Li, L. and Petrovsky, N. (2016). Molecular mechanisms for enhanced DNA vaccine immunogenicity. *Expert review of vaccines*, *15*(3), pp. 313-329.

Li, Yen-Der, et al. (2020). "Coronavirus vaccine development: from SARS and MERS to COVID- 19." *Journal of biomedical science* 27.1 1-23.

Loveday, E. K., Hain, K. S., Kochetkova, I., Hedges, J.F., Robison, A., Snyder, D. T., Brumfield, S. K., Young, M. J., Jutila, M. A., Chang, C. B. and Taylor, M. P. (2021). Effect of inactivation methods on SARS-CoV-2 virion protein and structure. *Viruses*, *13*(4), p. 562.

Mason, R.J., (2020). Pathogenesis of COVID-19 from a cell biology perspective. *European Respiratory Journal*, *55*(4).

McGuire, B. E., Mela, J. E., Thompson, V. C., Cucksey, L. R., Stevens, C. E., McWhinnie, R.L., Winkler,D.F., Pelech, S. and Nano, F. E., 2022. Escherichia coli recombinant expression of SARS-CoV-2protein fragments. *Microbial cell factories*, *21* (1), pp.1-13.

McIntosh, C. N. (2022). Re:"Consider this before using the SARS-CoV-2 pandemic as an instrumental variable in an epidemiologic study". *American Journal of Epidemiology*, *191* (1), pp.234-236.

Mossel, E.C., Wang, J., Jeffers, S., Edeen, K.E., Wang, S., Cosgrove, G.P., Funk, C.J., Manzer, R., Miura, T.A., Pearson, L.D. and Holmes, K.V. (2008). SARS-CoV replicates in primary human alveolar type II cell cultures but not in type I-like cells. *Virology*, *372* (1), pp. 127-135.

Nadin-Davis, S.A. (2020). Molecular epidemiology. In *Rabies* (pp. 143-193). Academic press.

Nag, K., Baray, J.C., Khan, M.R., Mahmud, A., Islam, J., Myti, S., Ali, R., Sarker, E.H., Kumar, S., Chowdhury, M.H. and Roy, R. (2021). An mRNA-based vaccine candidate against SARS-CoV-2 elicits stable immuno-response with single dose. *Vaccine*, *39*(28), pp. 3745-3755.

Nicholls, J. M., Butany, J., Poon, L.L.M., Chan, K. H., Beh, S.L., Poutanen, S., Peiris, J. M. and Wong, M. (2006). Time course and cellular localization of SARS-CoV nucleoprotein and RNA in lungs from fatal cases of SARS. *PLoS medicine*, *3*(2), p. e27.

Orenstein, W. A. and Ahmed, R. (2017). Simply put: Vaccination saves lives. *Proceedings of the National Academy of Sciences*, *114* (16), pp. 4031-4033.

Pardi, N., Hogan, M., Porter, F. *et al.* (2018). mRNA vaccines—a new era in vaccinology. *Nature Reviews Drug Discovery* **17,** 261–279 https://doi.org/10.1038/nrd.2017.243)

Parra-Lucares, A., Segura, P., Rojas, V., Pumarino, C., Saint-Pierre, G. and Toro, L. (2022). Emergence of SARS-CoV-2 Variants in the World: How Could This Happen?. *Life*, *12*(2), p. 194.

Plotkin, S. A. and Plotkin, S. L. (2011). The development of vaccines: how the past led to the future. *Nature Reviews Microbiology*, *9*(12), pp. 889-893.

Pollard, A.J. and Bijker, E. M. (2021). A guide to vaccinology: from basic principles to new developments. *Nature Reviews Immunology*, *21*(2), pp. 83-100.

Pustake, M., Tambolkar, I., Giri, P. and Gandhi, C. (2022). SARS, MERS and CoVID-19 : An overview and comparison of clinical, laboratory and radiological features. *Journal of Family Medicine and Primary Care*, *11*(1), p. 10.

Satarker, S. and Nampoothiri, M. (2020). Structural proteins in severe acute respiratory syndrome coronavirus-2. *Archives of medical research*, *51*(6), pp. 482-491.

Satarker, S. and Nampoothiri, M. (2020). Structural proteins in severe acute respiratory syndrome coronavirus-2. *Archives of medical research*, *51*(6), pp. 482-491.

Satarker, S. and Nampoothiri, M. (2020). Structural proteins in severe acute respiratory syndrome coronavirus-2. *Archives of medical research*, *51*(6), pp. 482-491.

Satarker, S. and Nampoothiri, M. (2020). Structural proteins in severe acute respiratory syndrome coronavirus-2. *Archives of medical research*, *51*(6), pp. 482-491.

Schaub, J. M., Chou, C. W., Kuo, H. C., Javanmardi, K., Hsieh, C. L., Goldsmith, J., DiVenere, A.M., Le,K. C., Wrapp, D., Byrne, P. O. and Hjorth, C. K. (2021). Expression and characterization of SARS-CoV-2 spike proteins. *Nature Protocols*, *16*(11), pp. 5339-5356.

Sun, J., Zhu, A., Li, H., Zheng, K., Zhuang, Z., Chen, Z., ... and Li, Y. M. (2020). Isolation of infectious SARS-CoV-2 from urine of a COVID-19 patient. *Emerging microbes & infections*, *9*(1), 991-993.

Vashishtha, V. M. and Kumar, P. (2021). Development of SARS-CoV-2 vaccines: challenges, risks, and the way forward. *Human Vaccines & Immunotherapeutics*, *17*(6), pp. 1635-1649.

Walls, A.C., Park, Y.J., Tortorici, M.A., Wall, A., McGuire, A.T. and Veesler, D. (2020). Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. *Cell*, *181* (2), pp. 281-292.

Wang, D., Fang, L., Shi, Y., Zhang, H., Gao, L., Peng, G., Chen, H., Li, K. and Xiao, S. (2016). Porcine epidemic diarrhea virus 3C-like protease regulates its interferon antagonism by cleaving NEMO. *Journal of virology*, *90*(4), pp. 2090-2101.

Wang, N., Shang, J., Jiang, S. and Du, L., 2020. Subunit vaccines against emerging pathogenic human coronaviruses. *Frontiers in microbiology*, *11*, p. 298.

Wang, Q., Qiu, Y., Li, J.Y., Zhou, Z. J., Liao, C. H. and Ge, X.Y., 2020. A unique protease cleavage site predicted in the spike protein of the novel pneumonia coronavirus (2019-nCoV) potentially related to viral transmissibility. *Virologica Sinica*, *35*(3), pp. 337-339.

Wang, X., Rcheulishvili, N., Cai, J., Liu, C., Xie, F., Hu, X., Yang, N., Hou, M., Papukashvili, D., He, Y. and Wang, P.G., 2022. Development of DNA Vaccine Candidate against SARS-CoV-2. *Viruses*, *14*(5), p. 1049.

Woolhouse, M., Scott, F., Hudson, Z., Howey, R. and Chase-Topping, M., 2012. Human viruses: discovery and emergence. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367* (1604), pp. 2864-2871.

Wu, A., Wang, L., Zhou, H. Y., Ji, C.Y., Xia, S. Z., Cao, Y., Meng, J., Ding, X., Gold, S., Jiang, T. and Cheng, G., 2021. One year of SARS-CoV-2 evolution. *Cell host & microbe*, *29*(4), pp. 503-507.

Xiao, F., Tang, M., Zheng, X., Liu, Y., Li, X. and Shan, H., 2020. Evidence for gastrointestinal infection of SARS-CoV-2. *Gastroenterology*, *158* (6), pp. 1831-1833.

Zhan, Y., Pang, Z., Du, Y., Wang, W., Yang, Y., Wang, W., Gao, G.F., Huang, B., Deng, Y. and Tan, W., 2020. NS1-based DNA vaccination confers mouse protective immunity against ZIKV challenge. *Infection, Genetics and Evolution*, *85*, p.104521.

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Strategies of SARS-CoV-2 Vaccine Production Using Nucleic Acid Based Vaccines and Conventional Methods

SARS-CoV-2 is the causative agent of the disease COVID-19 which emerged in 2019 and leads to a global pandemic. SARS-CoV-2 has genetic similarities with other human coronaviruses (MERS - CoV and SARS-CoV-1). Vaccination is the most effective method in preventing deaths, hospitalization, and the transmission of the infection. Conventional vaccine development usually lasts 10 to 15 years. Considering SARS-CoV-2 is highly contagious and highly transmissible, several innovative vaccines were developed in a short period of time to help combat this pandemic:

Vaccines are based on the viral genome and the whole structure as well to induce the host immune system. SARS-CoV-2 is cultured in lineage cells like VERO cells to produce inactivated and live attenuated vaccines, which are subsequently rendered inactive through chemical processes. Using RNA as mRNA and DNA as plasmids, genetic vaccinations are created.

The Spike protein is a potential element in SARS-CoV-2 vaccine designing. The viral recombinant spike protein is difficult to produce due to its size and its membrane fusion protein which is metastable and heavily glycolated. Therefore, the expression of SARS-CoV-2 recombinant protein fragments in *E. coli* is an effective approach in recombinant protein production. All vaccines were tested *in vivo* on animals and humans to regulate their safety and effectiveness. Since SARS-CoV-2 mutates, new strategies are required to generate new vaccines, while keeping all the preventive majors that have been implanted by governments and WHO.

Key words: SARS-Cov-2, Covid-19, pandemic, immune system, spike protein, vaccines platforms.

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