



REVIEW

COVID-19 and progress in therapeutic approaches: a narrative review

COVID-19 ve terapötik yaklaşımlardaki ilerleme: bir gözden geçirme

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Abstract

Coronavirus disease 2019 (COVID-19), scientifically known as severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) has adversely affect the respiratory system of humans and badly crushed the economies of most developed countries. This study aims to investigate the current COVID-19 situation and therapeutic approaches including non-viral vaccines, efficacy, safety, their trials, dosage protocols and mass vaccination established for COVID-19. A comprehensive literature search was conducted using PubMed, Scopus, Google Scholar, and MEDLINE databases. The title of the research articles were reviewed first, followed by the abstracts and finally the complete studies. This review critically focuses on the damaging effects of COVID-19 and its mutations, multiple variants, pharmacological approaches, and mRNA vaccines. COVID-19 is an emerging new variant with a different mutation and altered genomic structure. Different pharmacological and therapeutic approaches (interferon, oligonucleotides, anti-viral drug molecules, antibody peptides) have been made so far to combat COVID-19. Interferons are employed in COVID-19 treatment approaches owing to the role of Type I interferons in COVID-19 pathogenesis. Along with these therapeutic methodologies, companies from all over the world participated in the run to develop a vaccine for COVID-19. Vaccines were developed in less than a year for vaccinating humans on a mass level for the first time in world history. These vaccines target structural components of the virus, such as membrane proteins with a spike protein. This article provides valuable information for researchers regarding COVID-19 and progress in therapeutic approaches. This article will serve as a hallmark for understanding the COVID-19 mutation mechanism and strategies to combat these mutation points.

Öz

Bilimsel olarak şiddetli akut solunum sendromu koronavirus 2 (SARS-Cov-2) olarak bilinen koronavirus hastalığı 2019 (COVID-19), insanların solunum sistemini olumsuz etkilemiş ve çoğu gelişmiş ülke ekonomisini fena halde ezmiştir. Bu çalışma, mevcut COVID-19 durumunu ve viral olmayan aşıları, etkinliğini, güvenliğini, denemelerini, dozaj protokollerini ve COVID-19 için oluşturulan toplu aşlamayı içeren terapötik yaklaşımları araştırmayı amaçlamaktadır. PubMed, Scopus, Google Scholar ve MEDLINE veritabanları kullanılarak kapsamlı bir literatür taraması yapılmıştır. Araştırma makalelerinin başlığı önce gözden geçirildi, ardından özetler ve son olarak çalışmaların tamamı incelendi. Bu derleme eleştirel olarak COVID-19'un zararlı etkilerine ve mutasyonlarına, çoklu varyantlarına, farmakolojik yaklaşımlarına ve mRNA aşılarına odaklanmaktadır. COVID-19, farklı bir mutasyona ve değiştirilmiş genomik yapıya sahip, ortaya çıkan yeni bir varyanttır. COVID-19 ile mücadele etmek için şimdiye kadar farklı farmakolojik ve terapötik yaklaşımlar (interferon, oligonükleotitler, anti-viral ilaç molekülleri, antikör peptitleri) yapılmıştır. Tip I interferonların COVID-19 patogeneziindeki rolü nedeniyle COVID-19 tedavi yaklaşımlarında interferonlar kullanılmaktadır. Bu terapötik metodolojilerin yanı sıra dünyanın dört bir yanından şirketler COVID-19 aşısı geliştirme çalışmasına katıldı. Aşılar, dünya tarihinde ilk kez kitlesel düzeyde insanları aşılama için bir yıldan kısa bir sürede geliştirildi. Bu aşılar, başak proteinli zar proteinleri gibi virüsün yapısal bileşenlerini hedefler. Bu makale, COVID-19 ve terapötik yaklaşımlardaki ilerleme konusunda araştırmacılar için değerli bilgiler sunmaktadır. Bu makale, COVID-19 mutasyon

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Received: 08.12.2023 Accepted: 08.03.2024

Keywords: COVID-19, vaccine, therapy, replication

mekanizmasını ve bu mutasyon noktalarıyla mücadele stratejilerini anlamak için bir ayırt edici özellik görevi görecektir.

Anahtar kelimeler: COVID-19, aşı, tedavisi, replikasyonu

INTRODUCTION

Coronavirus disease 2019 (COVID-19); a variant of coronavirus, originated in Wuhan, China on December 31, 2019, and has since spread throughout the country and to numerous other countries, resulting in onward t. Genomically, it is associated with the genera Beta Coronavirus order Nidovirales; it is a zoonotic virus, and the main target of this virus is humans. It is believed to be originated from bats, as 96 % of nucleotides are similar to bat severe acute respiratory syndrome (SARS)-like coronavirus¹. The virus normally induces flu-like symptoms such as fever, cough and cold, with severe cases potentially leading to respiratory difficulties². Additionally, it is believed to induce inflammation, heart injury, cardiac failure, and multi-organ dysfunction. The risk is higher in elderly people and patients with co-morbid conditions³. The person-to-person spread rate of the virus is 2.6, indicating its spread at an exponential rate^{4,5}. Generally, outbreaks of coronaviruses have affected the human population for fifty years, causing mild to moderate respiratory infections. However, in the 21st century, three out of seven coronaviruses emerged as a major threat, with COVID-19 being the most significant in terms of its widespread impact⁶ there has been a 52% increase in new cases globally over the preceding 28-day period, with over 850,000 new cases reported. However, the number of new deaths decreased by 8% compared to the previous 28-day period, with over 3,000 new fatalities reported. As of 17 December 2023, over 772 million confirmed cases and nearly seven million deaths have been reported globally⁷. This study aims to investigate the current COVID-19 situation and therapeutic approaches, including non-viral vaccines, efficacy, safety, their trials, dosage protocols and mass vaccination established for COVID-19.

MATERIALS AND METHODS

We conducted a comprehensive literature review using PubMed, Scopus, Google Scholar, and MEDLINE databases. The title was examined first, followed by the abstract and finally the complete study. This review primarily focuses on COVID-19,

it's damaging effects, mutations, multiple variants, pharmacological interventions, and mRNA vaccines. Therefore, this study is based on a narrative literature review, therefore, approval from the ethics committee was not required.

RESULTS

The findings of the literature review on COVID-19 are presented and discussed under various headings as;

Route of transmission

COVID-19 is primarily spread by the frequent emission and exposure of tiny respiratory droplets that are emitted during talking, coughing and sneezing of a diseased person⁸. These salivary droplets are loaded with a large amount of virus that remains in the air for some time and hence spread infection⁹. COVID-19 enters the human body through the mouth, eyes and nose either by direct contact with the salivary droplets of an infected person or by touching the infected areas¹⁰. Whereas the viral spread by touching the contaminated areas is highly dependent on the humidity and temperature of the atmosphere.

Super spreader

An engineer in India came to his village for his marriage, and most of the villagers attended his marriage in May 2020. He died of fever after two days. When the participants were tested for covid, 79 were found positive. These participants spread the disease to 24 people with whom they come in contact with. The studies show that a typical COVID-19-infected or carrier person can infect two to three persons on average. The epidemiologists call these persons "super spreaders"¹¹. So, the reproduction number is 2 to 3. The best remedy to overcome this global pandemic is maintaining a physical and social distance of 1.5 to 2 meters, using a multilayered mask, and washing hands with plenty of water and soap for at least 20 to 40 seconds. Traveling in outside countries, quarantine and isolation are the best ways to avoid the spread of COVID-19 globally¹¹.

COVID-19 Stability: COVID-19 is stable under harsh conditions, extreme pH and high temperatures. The virus was found by Japanese researcher Professor Eiji Haramoto in drainage water. Samples of sewage water were collected, and PCR was done, the sample was found to be COVID-19 positive. The people in the concerned areas were found to be COVID-19 positive. The same striking results were also found in the United States and France. These studies suggest that to avoid further waves of infection of COVID-19, it's necessary to properly dispose of sewage water.

Genomic structure: COVID-19 is an enveloped virus, that contains a single-stranded RNA (positive-sense) that is linked with a nucleoprotein encapsulated in a capsid containing matrix proteins. It contains 29,903 nucleotides in its structure. The main COVID-19 proteins are spike glycoprotein (S), nucleocapsid protein (N), and membrane glycoprotein (M)¹². S protein is cut by the host protease enzyme into its two subunits, S1 and S2.

These subunits are mainly responsible for the fusion of the host and viral membrane and assist the entry of viral genetic material into the host cell¹³. Unlike other Coronaviruses, COVID-19 encodes a supplementary glycoprotein that shows all the characteristics of hem agglutination (HE) and acetyl esterase enzyme¹⁴. Generally, COVID-19 consists of 14 ORFs genes (open reading frames) in its genomic structure possessing 27 proteins. ORF arranges themselves as replicate and protease enzymes. ORF 1 encodes two polyproteins; ORF 1a and ORF 1b encode pp1a and pp1b, which further produce 16 proteins similar in all the coronaviruses of the same family. ORFs 10 and 11 encode four major structural proteins. Others are responsible for encoding matrix protein (M), small envelope protein (E), spike glycoprotein (S) and nucleocapsid protein (N). The virus interferes with host immunity with its accessory proteins¹⁵. Single-guided RNAs (sg RNAs) of the COVID-19 virus are responsible for the translation of all the accessory and structural proteins¹.

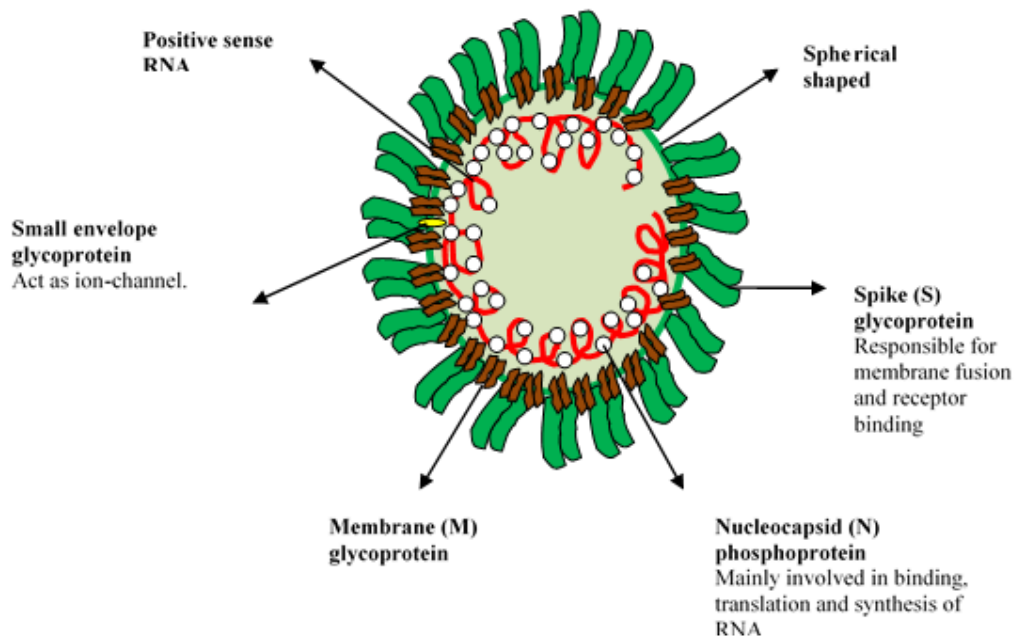


Figure 1. Structure of COVID-19.

Viral replication inside the host cell

COVID-19 identifies angiotensin-converting enzyme 2 receptors on the host cell and utilizes its spike proteins for binding to the receptor site present on the host cell surface. This binding is mediated by the

receptor binding domain present on the S1 subunit, this interaction results in the binding of the S2 subunit with the host cell surface¹⁶. After binding to the host cell COVID-19 injects its genetic material, replicates and produces multiple copies of the parent virus.

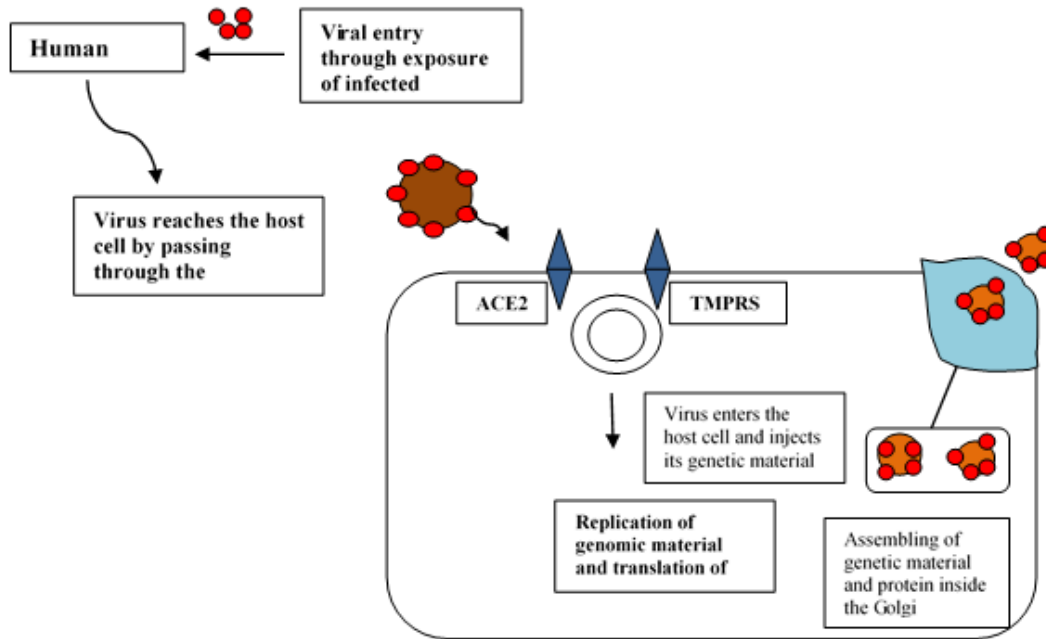


Figure 2. Diagrammatic representation of COVID-19 replication.

Mutation

A mutation is a particular alteration in the genomic sequence of a virus when compared with the already approved and accepted sequence. Such viral particles with these genomic alterations are called variants. These alterations can be insertion, deletion, or point mutations and are more beneficial than the parent ones.

COVID-19 resembles SARS-CoV and binds with the host receptor site by its spike proteins. Receptor binding proteins of COVID-19 share 89.2% similarity with SARS-CoV. These RBD and hydrophobic pockets conserved in both virus types. Research studies also reveal that the binding free energy of the interaction between SARS-CoV binding proteins and host receptor ACE-II is lower

than the binding energy of SARS-CoV receptor binding proteins and host angiotensin converting enzyme-II interaction. This strong interaction provides a reason for the more infectious nature of COVID-19¹⁷. Spike proteins present on the cell surface of the COVID-19 virus determine infectivity, functionality, solubility of virus particles and stability and are important for the survival of the virus. The total energy of the spike proteins is lower and thus it provides more stability to COVID-19 and can bear high temperatures as compared to SARS-CoV¹⁸.

It is assumed that COVID-19 is derived from bat Coronavirus because of mutation in protein S (spike glycoprotein), nucleocapsid N protein and in S1-S2 junction¹⁹. Spike protein's RBD is believed to be the most mutable part of the virus genome. Six amino acids present in the receptor binding domain of

Coronaviruses are crucial for binding these viruses to the host cell surface receptors, in SARS Cov-2, five out of these six amino acids are different providing a chance for more efficient binding with the host receptor site. The genome sequence of Rhinolophus

affinis bat indicates that COVID-19 is 96 % similar to it with a divergence in its receptor binding domain²⁰. Hence all Coronaviruses are believed to be derived from the same ancestors.

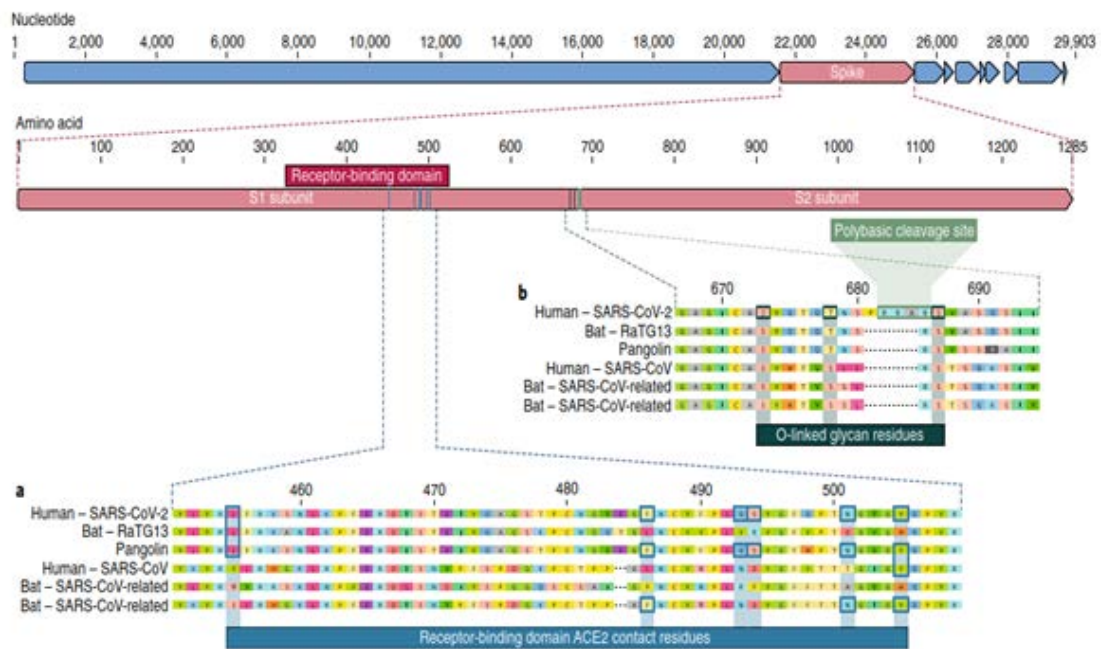


Figure 3. COVID-19 genomic structure

COVID-19 variants

When a virus infects people by circulating in a population, the chances of mutation increase. The more a virus spreads in the population, the more it gets a chance to replicate and change. It is the characteristic of a virus. These mutations alter the transmission of the virus, its severity (a variant might be more lethal than its ancestor or less lethal), can escape from the immune system of the host more efficiently, and vaccine efficacy. Variant B.1.1.7 (alpha variant) was first identified in the United Kingdom in 2020, this variant accounts for 11 % of all the COVID-19 cases in South Africa, which increased up to 60% in the first week of November and up to 87 % in the first week of December, this variant has been spread to 93 countries till February 2021. This variant possesses 23 mutations and 17

altered amino acids with 13 non-synonymous point mutations, 4 deletions and 6 synonymous point mutations (C913T, C14676T, C15279T, C16176T, C5986T), and in the M gene (T26801C). Alpha variant increases the transmissibility of the virus up to 43 % to 82 %. 501Y.V2 variant was first observed in South Africa on December 18 and has been observed in 45 countries after this, 501Y.V2 variant is more transmissible than its precursor and infected the population 50 % more quickly than SARS Cov-2, P.1 variant with 35 mutations and 17 altered amino acids was found in Brazil on 12 January 2021. The gamma variant can cause infection in persons who have already been infected by different strains and have strong immunity against coronavirus²¹. The reason for this alteration in all variants is the replacement of asparagine with Tyrosine in spike protein’s receptor binding domain at position 501. In

the case of beta and gamma variants, two additional alterations are observed, E484K and K417N/T. These mutations result in increased affinity and

binding of the virus with a binding site of the host cell surface (Angiotensin converting enzyme-II).

Table 1. Current variants of concern of COVID-19

Variant	Lineage	First Detected in	Country	Point of concern
VOC 202012/01	B.1.1.7	Sep 2020	United Kingdom	Increased transmissibility, increased severity
501 Y.V2	B.1.351	Oct 2020	South Africa	Possible reduction of vaccine effectiveness, Increased transmissibility, increased severity
P.1	P.1	Dec 2020	Brazil	Possible reduction of vaccine effectiveness, Increased transmissibility, increased severity
Variants, common names and phenotypic changes				
Variant	B.1.1.7	B.1.351	B.1.1.28.1	B.1.617.2
Common Name	Alpha	Beta	Gamma	Delta
Phenotypic Change	Increase transmission	Increase transmission and virulence	Decrease neutralization Increase transmission and virulence	Decrease neutralization, Increase transmission

Treatment strategies

Adaptive mutations in the structure of COVID-19 made it more pathogenic and vaccine development difficult²². Research studies indicate that lipid molecules present in Coronavirus are primarily responsible for the entry of the virus to the host cells by interacting with the host lipids, thus targeting the host lipids can be used as an antiviral strategy for combating COVID-19²³. Targeting of the COVID-19 enzymes like papain-like protease (PLpro), structural proteins, RNA-dependent RNA polymerases (RdRP) and 3C-like protease can be employed for designing specific anti-viral drugs²⁴.

THERAPEUTIC AND PREVENTIVE APPROACH TO COVID-19

Although there is no proper treatment for COVID-19 has been found yet it can be managed by using immuno-stimulators, antimalarial drugs, antiviral drugs and other supplementary therapeutic options²⁵. In comparison to an increase in mortality and

increased incidence of COVID-19, the treatment options are limited to treating COVID-19 patients. New treatments are time-consuming, as it will take time to develop new medicines to establish their safety and efficacy profile. Thus, the existing multipurpose treatments can provide immediate treatment options for the present pandemic. These include broad-spectrum antivirals as an adjunct and to provide supportive treatment. Though these are not the definitive treatments for COVID-19 but provide promising results in early-stage investigational studies. In a study conducted in Shenzhen, Favipiravir showed more rapid mean time clearance than other antivirals²⁶.

Therapeutic treatments intend to treat a disease, increase the chances of survival of patients and minimize the stay duration of patients in the hospital. Vaccines are aimed at protecting mankind by generating proactive immunity. In the present situation, where COVID-19 has become a global pandemic spreading rapidly, with limited health

facilities, restricted ventilators and limited hospital capacity, prophylactic vaccines are crucial.

Table 2. Summarized form of drugs that are under investigation for COVID-19 treatment.

Drug classes	Drugs
Anti-viral agents	Remdesivir, Ribavirin, Novaferon, Lopinavir, Nirmatrelvir-ritonavir, Molnupiravir
Other drugs that can be used as antiviral agents	
Antibiotic	Doxycycline
Anti-malarial agents	Chloroquine and hydroxyl-chloroquine
Immunomodulators	IL-6 inhibitors, steroids, interferon
H2-receptor antagonist	Famotidine
Gold-containing drugs	Auranofin
Serine protease enzyme inhibitors:	Nafamostat, Camostat
Adjuvant therapies for COVID-19	Vitamin-C Anticoagulants Convalescent plasma
Vaccines	

ANTIVIRAL DRUGS

Majority of the COVID-19 patients have mild to moderate respiratory tract infections and can be treated even without using any antiviral drugs, whereas some patients may develop severe acute respiratory syndrome (SARS) and need proper treatment. In this case, antiviral therapy is required to reduce the time span and severity of the disease²⁷. Commonly used antiviral drugs are; Remdesivir, Lopinavir/ritonavir, Novaferon, Favipiravir, etc.

Remdesivir

Remdesivir is a broad-spectrum nucleoside analogue that comes under the category of polymerase inhibitors. It has a small molecular structure that is effective against RNA viruses such as; MERS-Cov, Hendra viruses, SARS-Cov, Ebola virus and Nipah virus. Remdesivir is a precursor of adenosine analogue. After administration, this prodrug is converted into its active form, nucleoside triphosphate and shows antiviral activity against RNA viruses including COVID-19¹⁰. Being an RdRp inhibitor (RNA-dependent RNA polymerase), it inhibits the replication of COVID-19. Its active metabolite, nucleoside triphosphate (NTP) utilizes ATP which is crucial for the development of viral RNA. Hence RNA is not fully synthesized and this

premature RNA further restricts the growth and development of viral genome¹⁰.

Ribavirin

Ribavirin is a broad-spectrum anti-viral agent that acts as a nucleoside analogue and inhibits the production of viral genomic material²⁸. It acts as a precursor of a drug and is converted into its active form after metabolism. Its active form is similar to purine RNA nucleotide that hinders the metabolism of RNA which is required for viral proliferation and replication²⁹.

Novaferon

Novaferon is an artificially synthesized protein that is made up of 167 amino acids, based on DNA shuffling technology. It inhibits the spread of COVID-19 in normal cells by restricting viral replication in contaminated cells. Hence, it is considered a potential antiviral drug for COVID-19 treatment^{10,30}.

Lopinavir

Lopinavir is a protease enzyme inhibitor and is used in combination therapy for people infected with the Human immunodeficiency virus. Nowadays this combination therapy has also shown potential for COVID-19 treatment. Lopinavir restricts the

replication and release of the virus by inhibiting chymotrypsin-like protease enzyme 3CLpro. Hence it is under observation for COVID-19 treatment³¹.

Nirmatrelvir-ritonavir

The Food and Drug Administration (FDA) has approved ritonavir-boosted nirmatrelvir for the treatment of mild to moderate COVID-19 in adults at high risk of developing severe COVID-19. The COVID-19 Treatment Guidelines Panel advises nonhospitalized people with mild to moderate COVID-19 who are at high risk of disease progression to take 300 mg of nirmatrelvir and 100 mg of ritonavir (Paxlovid) orally twice a day for five days³².

Molnupiravir

Molnupiravir is an oral prodrug of beta-D-N4-hydroxycytidine (NHC), a ribonucleoside that has been demonstrated to have antiviral activity against COVID-19 in vitro and clinical trials³³. When ritonavir-boosted nirmatrelvir and remdesivir are not available, feasible to use, or clinically appropriate, the COVID-19 Treatment Guidelines Panel recommends using molnupiravir 800 mg orally twice daily for five days as an alternative therapy in non-hospitalized patients aged ≥ 18 years with mild to moderate COVID-19 who are at high risk of disease progression; treatment should be started as soon as possible and within five days of symptom onset³⁴.

Some other classes of drugs that are used as Anti COVID-19 agents are;

ANTIBIOTICS

Doxycycline

The growth and survival of COVID-19 depend on its binding with metalloproteases (MMPs). Doxycycline inhibits this interaction by chelating zinc from MMPs which in turn restricts the spread of COVID-19³⁵.

H2-receptor antagonists:

Famotidine

Famotidine is an antihistamine drug that binds preferably with H2-receptors and reduces the release of gastric discharge. It is designed in such a way that it inhibits the proliferation and multiplication of protease enzymes of COVID-19. However, its

effectiveness against COVID-19 is still under observation³⁶.

Antimalarial drugs

Chloroquine and hydroxychloroquine

Chloroquine and hydroxychloroquine utilize several pathways to restrict viral pathogenicity. It inhibits the antigen-host interaction by interfering with surface receptors. It also modifies the PH of endosomes and impedes the initial step of genomic replication. Additionally, it inhibits the interleukins (ILs) such as IL-6 and IL-1beta and decreases the inflammation caused by COVID-19³⁷.

Gold-containing drugs

Auranofin

Auranofin is a complex gold-containing substance that shows an affinity for lipid molecules. These organogold compounds reduce cytokine release and activate cell-mediated immunity (Drug Bank). They are clinically approved for the treatment of rheumatoid arthritis as an anti-neoplastic and anti-inflammatory agent. Auranofin activity against COVID-19 is under investigation. Research suggests that it restricts the replication and proliferation of COVID-19 even at low concentrations. However, further investigation is required to prove its clinical effectiveness against COVID-19³⁸.

Immunostimulatory drugs

Corticosteroids

Corticosteroid acts as an anti-inflammatory agent that is mainly used in the treatment of pneumonia. It decreases the oozing fluid inside the lung tissues, treats bronchoconstriction by reducing systemic inflammation, and prevents respiratory failure³⁹. This immunomodulatory agent is employed in critical and hyper-inflamed COVID-19 patients due to its anti-inflammatory effect⁴⁰.

Interleukin-6 Inhibitors

Tocilizumab: Tocilizumab is highly specific for IL-6 receptors, binds with them and blocks their function. As a result, the release of the inflammatory mediator, interleukin is inhibited which in turn reduces inflammation. Due to its anti-inflammatory effect, it is considered safe and effective for the treatment of rheumatoid arthritis. Its effectiveness in the treatment of COVID-19 is still under investigation.

Interferon-1

Interferons are cytokines, which show antiviral activity by producing intracellular antiviral proteins⁴¹. These proteins restrict the viral multiplication process and stimulate the macrophages, Natural killer cells and cytotoxic T lymphocytes (CTL). As a result, the cell-mediated immunity is activated which further controls the COVID-19 replication and proliferation inside the host cell⁴².

To understand the effect of interferon manifestation in COVID-19 patients, a study was conducted on the skin samples of ten COVID-19 hospitalized patients with moderate to severe illness⁴³. The results showed that the genes regulating the macrophage function were upregulated. These include the genes for interleukin 32, chemokines that attract monocytes, and pro-inflammatory cytokines (Interleukin 1A, 1B, and 6). Moreover, the number of CD163+ macrophages, T cells, and neutrophils, and the number of pathophysiological features of COVID-19; macrophages that produce IFN- β were also increased. The other endotheliopathies; swelling of endothelial cells, loss of integrity of endothelial cells, and caspase -3 were also observed.

STING activation and H-151 treatment in COVID-19 patients

COVID-19 patients displayed increased levels of cGAMP, the Stimulator of interferon genes (STING) was also observed in COVID-19 lesions⁴⁴. Damaged mitochondria were also observed in endothelial cells of skin in COVID-19 patients. These findings indicate the upstream of STING caused by cGAS in endothelial cells. Thus COVID-19 can induce mitochondrial dysfunction in COVID-19 patients, which in turn activates the cGAS-STING pathway by releasing endogenous mitochondrial DNA. H-151, an inhibitor of STING was found to be effective in interferon gene activation reduction. The cytopathic effect induced by the COVID-19 virus was also reduced in individuals when treated with H-151.

In-vivo testing for STING activation in COVID-19 patients

For the investigation of the STING effect in COVID-19 patients, the experiment was performed in vivo on K18-hACE2 transgenic mice (as they are considered as most susceptible species to Coronavirus). The mice were divided into two groups; one group was given an H-151 inhibitor, before COVID-19 infection. The group injected with

the H-151 inhibitor showed a significant reduction in inflammatory cells. H-151 inhibitor also reduces cell death. The results showed that STING is a contributor to lung pathology. As a therapeutic agent, H-151 was found to be effective in reducing interferon expression^{45,46}, pro-inflammatory genes³⁵, lung injury, NF- κ B activity, and chemokines⁴⁷. However, H-151 showed no effect on viral replication. H-151 is believed to show its effect through STING inhibition, thus the results confirm that STING doesn't have any effect on viral replication⁴⁶. H-151 is therapeutically effective in the prophylactic and therapeutic treatment of the ongoing disease. This study discovered a novel mechanism of immunopathology involving the cGAS-STING pathway that induces endothelial dysfunction, and the production of interferons. Endothelial dysfunction is induced by the accumulation of mitochondrial DNA in the cytosol. The studies also reveal the vascular damage induced by COVID-19 pathology.

Serine protease enzyme inhibitors: Nafamostat, Camostat

The protease enzyme of the host cell, TMPRSS2 is crucial for the efficient binding of viral spike protein receptor with ACE2 receptor. Serine protease enzyme inhibitors such as; Nafamostat and Camostat inhibit TMPRSS2 and restrict the entry of COVID-19 inside the host cell¹⁰.

Other supplementary treatments: Some other adjuvant therapies for COVID-19 are;

Plasma of healthy patient

Administration of convalescent plasma which is loaded with COVID-19-specific antibody (IgG) is found to be the most effective treatment of severely ill patients. This transfusion will surely help our immune system to fight against COVID-19⁴⁸. For COVID-19, it's important to investigate, for how long a person will be safe from re-attack by the virus. Studies revealed that after a person gets infected by the virus, he can lose immunity against the virus within months. A study was conducted by researchers, in which 90 patients participated, and 60 % of the patients showed strong antibodies in the first weeks. After 3 months, the antibodies were found in 16.7 % of the patients. The present studies also reveal that people will need booster doses for the prevention of COVID-19.

Vitamin C

Vitamin C is effective in reducing inflammation and oxidative stress and in improving the immune system⁴⁹. The expert panel of the Shanghai Medical Association suggests that the daily 100-200mg/kg intravenous (IV) administration of vitamin C is found to improve oxygen levels in COVID-19 patients⁵⁰.

Anti-coagulants

heparin/low molecular weight heparin: Although there is no clotting occurring in COVID-19 patients, however, some patients may develop small fibrin clumps in the lungs. Viral infection stimulates tissue factor pathway, toll-like receptor and enhances the level of von Willebrand factor which in turn form fibrin clots³². Due to the anti-inflammatory and anticoagulant activity of heparin, it is not only considered to be effective in COVID-19 patients but also useful for those patients who developed sepsis-induced coagulopathy due to COVID-19⁵¹.

Proteins to attach with the angiotensin-converting enzyme II receptor, (ii) proteases that help spike proteins to bind with the host receptor.

These components can be the potential targets for COVID-19 drug development. COVID-19 cannot be treated by taking any arbitrary medicine, herbal products or multivitamins. The only prevention against COVID-19 is the vaccine.

MECHANISMS OF VACCINES

The introduction of a vaccine in the body acts as a pathogen-antigen. The body recognizes this vaccine as a foreign antigen particle and triggers the body's immune response without causing the disease. The body destroys this foreign antigen and makes memory cells, so in case of a future attack by the pathogen, the body is ready for a quick and effective defensive response. A vaccine must have the following specifications to be categorized as an ideal vaccine. (i) Must effectively prevent the disease (ii) must reduce the severity of the disease (iii) provide long-lasting protection against the disease. (iv) Vaccine administration doses should be minimum (v) provide maximum antigen exposure without causing the disease. (vi) Must have minimal side effects (vii) vaccine should not be that costly and can be affordable by the public⁵². The aim of COVID-19

vaccination is the production of a strong immune response, where antibodies can bind to the spike S proteins of the virus to inhibit its binding with the host cell surface⁵³.

The body recognizes the viral structural proteins (protein M, protein S and protein E) as attackers, and makes specific antibodies against these attackers⁵⁴. Viral S protein is the main protein that is responsible for triggering the body's immune response. Neutralizing antibodies bind to the S1 and S2 structural areas of the viral membrane, thus effectively inhibiting the binding of the virus to host cells⁵⁵. The body also prepares T cells for long-term immunity against the virus⁵⁶. Antibody development takes a minimum of 5 days after the attack of the virus and onset of symptoms, with IgM antibodies appearing earlier than IgG antibodies taking a minimum of 7 days⁵⁷. All countries from all over the world contribute to COVID-19 vaccine development with the European Union \$8 billion, and Japan 800 million dollars. Spain and Italy, in 2020, remained as hardest hit and the biggest pandemic center for coronavirus contributed 100 million euros. Germany granted 525 million euros. 378 million euros, 60 million euros, 192 million euros were given by Switzerland, Israel, and Netherland correspondingly. The purpose of this funding was to make vaccine available for every nation, not for the rich and the most developed countries.

STRATEGIES FOR VACCINE DEVELOPMENT

Vaccines require post-treatments after manufacturing. Nucleic acid-based vaccines (mRNA and DNA) require vehicles for sufficient cellular uptake and delivery of antigens into the host cells. In protein-based vaccine production, protein antigens are synthesized and characterized. These antigens are in soluble protein form, thus recombination techniques are employed to maintain their antigenic character and for folding. However, mRNA and DNA vaccines are safe and these vaccines trigger a weak immune response, thus requiring immunostimulators (in the form of antigen-presenting cell receptors binding ligands with them) for immune response enhancement. They bind with receptors of antigen-presenting cells thus activating downstream pathways for strong immune response.

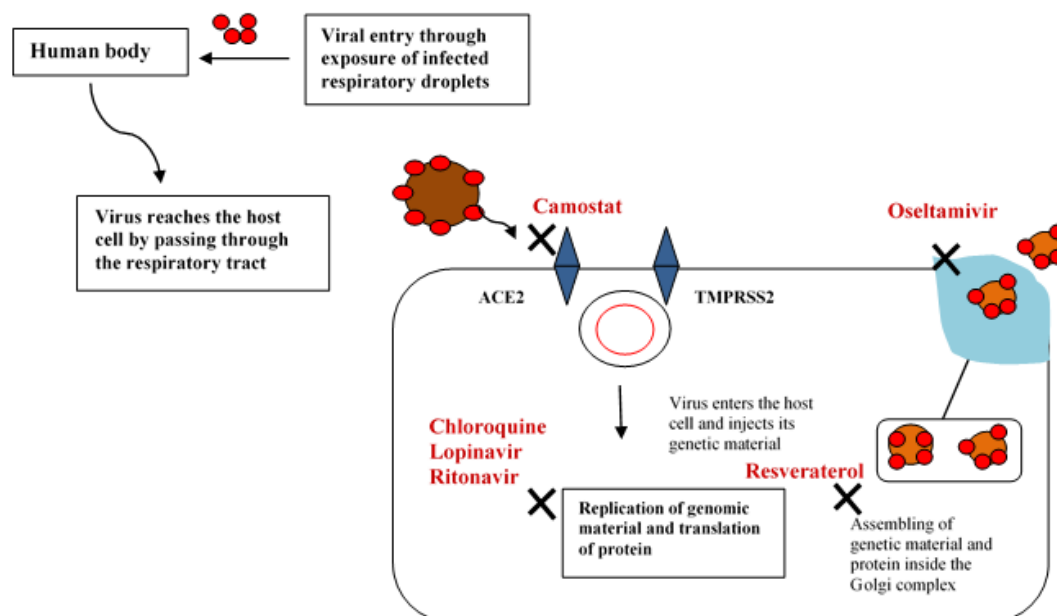


Figure 4. Schematic representation of viral replication and inhibitory site of potential drugs.

Need for a vaccine: COVID-19 utilizes its two components to interact with the host cell surface (i) spike

POSSIBLE VACCINE TYPES

Non-viral vaccines

Nucleic-acid vaccines

To produce nucleic acid-based viral proteins, viral mRNA or DNA is inserted into vaccinated individuals to force them to make viral proteins. In situ is another method to produce viral proteins. The vaccines produced by this method are produced speedily having more stability and safety. Moreover, these vaccines trigger the response of CD8+ cytotoxic T cells⁵⁸. In-vitro transcription technique can be employed for the production of mRNA vaccines⁵⁹. Messenger RNA-based vaccines for COVID-19 include Moderna (US) and BioNTech-Pfizer (Germany). They are supposed to neutralize viral Spike proteins and thus inhibit viral binding to the host cells⁵⁸. DNA vaccines are more stable than mRNA vaccines, but mRNA vaccines supersede DNA vaccines as they are not mutagenic, and their

immunogenicity can be modified⁶⁰. The mRNA and DNA vaccines are non-viral vaccines that are administered by using vaccine delivery technologies.

mRNA vaccines

The mRNA vaccines are the most reliable and the most advanced newly emerged technology for COVID-19. The mRNA vaccines are not genotoxic because they don't incorporate into the host's genome and thus are safe as compared to DNA vaccines⁵⁸. Unlike inactivated or live attenuated vaccines, mRNA vaccines are easy to manufacture because of fewer chances of contamination and decreased quality control tests⁶¹. Lipid-based mRNA is biodegradable and induces a strong immune response with safety⁶². The mRNA can be manufactured in a small period in billions of doses⁶³. These vaccines can be easily tolerated and produce efficient responses. However, mRNA formulation has some technical problems; unstable mRNA formulations can be easily degraded by RNases, and cannot be easily entered into the cells for translation of these mRNAs into protein. They can activate innate immunity and can induce inflammatory responses in the recipient's body. The vaccines require cold storage for distribution. To overcome

these hurdles, advanced mRNA technologies are required to ensure thermal stability, effectiveness and safety. In the history of vaccine development, within the shortest period, within 88 days, the dose was administered to the first human for clinical trial. mRNA when tested on non-human primates was found to induce CD4 cells, inducing a strong immune response in them. Both for Moderna's trial and Pfizer mild side effects were observed.

Design considerations for mRNA vaccines

The delivery vehicle for mRNA delivery into the cells is lipid nanoparticles. Lipid nanoparticles are solid structures of solid, which are composed of four structural components, ionizable lipids for complex formation, cholesterol for stabilization of the nanoparticles, PEG lipids for avoiding non-specific interactions, and helper phospholipids for the release of mRNA into intracellular cells. Lipid nanoparticulate matter carries mRNA inside it and increases the delivery of these vaccines into the cytosol for translation. LNP (lipid nanoparticles stabilize mRNA and protect it from degradation in intercellular space and can be synthesized in large amounts.

Developing lipid nanoparticles for mRNA delivery

For the delivery of mRNA into the host cell, lipid nanoparticles should have the following features, appropriate ionizable lipid bearing neutral, or having slightly positive charge owing to their safety profile⁶⁴. In this condition, they encapsulate mRNA having covid antigens at lower pH. Moreover, they maintain reduced non-specific interactions because of their neutral nature in intercellular space. After the mRNA-bearing lipid nanoparticles enter the cells, the low pH nature of intracellular cells charge the lipid particles and thus release mRNA in the cytosol. For the best delivery of mRNA, appropriate ionizable lipids should be chosen⁶⁵. Ionizable lipids that can be employed include DLin-MC3-DMA⁶⁶, DLin-KC2-DMA⁶⁷, cKK-E12⁶⁸, C12-200⁶⁹, Acuitas⁷⁰, ssPalmE⁷¹, L319⁷². These ionizable lipids having super performance are good candidates for mRNA vaccine formulation.

Optimization of mRNA encoding delivery vehicles

The other most important factor for formulating mRNA is the use of optimized PEG, cholesterol, and phospholipids with the maintained relative ratio.

These factors are important for the effectiveness and performance of mRNA vaccines. To make the process optimized, the design of experiment methodology is employed including screening and fractional factorial designs for determining lipid ratio and lipid to mRNA ratio. This increases the efficacy of the vaccine. In a trial, erythropoietin mRNA was enclosed in C12-200 for optimization which results in seven times increased efficacy as compared to the naked mRNA.

Liposomes having positive charge can also be employed as a delivery vehicle for mRNA. They include lipofectamine containing DOSPA and DOPE, and liposomes having DOTMA and DOTAP in their structure. In addition to conventional lipid particles, cationic oligo polymers can be employed as delivery vehicles. For instance, charge-altering releasable vehicle (CART). After the delivery of mRNA into intracellular space, CART itself undergoes degradation and releases mRNA into the cellular medium which results in mRNA translation into protein. Likely, amino esters have their applications in mRNA delivery. An example of such a formulation is the mixture of beta-amino esters and cholesterol, 1, 2-distearoyl-sn-glycero-3-phosphocholine, polyethylene glycol 2000, encapsulating mRNA for intracellular delivery. Micelles and emulsions are the other tools for mRNA delivery into host cells⁷³. Examples include nano micelle PEG-PAsp (TEP)-Chol for pancreatic cancer treatment. Micelles PEI-stearic acid conjugates encapsulating mRNA are found to be potential for HIV treatment⁷⁴.

Overall, for the optimization of mRNA vaccine delivery, the following considerations are crucial. The delivery system should be thermally stable and should allow storage and transportation at room temperature. It must have adjuvant properties, should have a broad spectrum for several pathogens along with specificity and it must provide a humoral and cellular immune response. The final packaged form must have effective quality packing, properly protecting the encoded mRNA and should have low cost, affordable for everyone and for all nations, to ensure that distribution is not restricted to wealthy and highly developed countries.

DNA vaccines

DNA vaccines are formulated by encapsulating vaccine antigens in bacterial circular extracellular DNAs⁷⁵. In DNA vaccines, mRNA delivery vehicle

plasmid enters into the nucleus of the infected cells (antigen-presenting cells). After the entry of the vaccine into the intracellular space, plasmid encapsulated genes (mRNA) express themselves, synthesize copies of foreign antigens and thus initialize humoral and cellular response against the pathogens⁷⁶. DNA vaccines are easy to manufacture, are safe with fewer adverse effects and are thermally stable without any risk of denaturation and biological activity loss and they can be easily transported. Like mRNA vaccines, DNA vaccines also require a short period for their manufacturing and a lot can be manufactured in 2 to 4 weeks⁷⁷. However, DNA vaccines have potential disadvantages as well. They can activate cancer genes and are mutagenic because of plasmid incorporation with the host genome. Owing to this property, DNA vaccines can induce autoimmune responses and long-term inflammation. DNA vaccines are novel advancements and primary vaccine technologies in the field of non-viral vaccines for human use. The effect of DNA vaccines has been evaluated against the COVID-19 virus, expressing 6 kinds of spike proteins of the virus in rhesus macaques⁷⁸. In the trial, 5 mg of the vaccines were administered to non-humans by intramuscular route at 0, 3rd and 5th week. These animals develop antibodies, spike proteins and receptor binding protein antibodies and Th1-based immune response⁷⁸. After animals got vaccinated, they were infected by COVID-19 through the nasal route, and the virus load was found to decrease by 4 fold in the nasal mucosa. After trials on non-human primates, a Phase-I DNA vaccine clinical trial was conducted on healthy adults. Vaccines proved potential efficacy and safety in these healthy individuals⁷⁹. DNA vaccines are under clinical trials by different research and development groups including Genexine, Symvivo, Zydus Cadila, and AnGes. INO-4800 was developed by Inovio Pharmaceutical targeting the spike protein of the virus⁸⁰.

Inovio Pharmaceutical developed INO-4700 when clinically trialed indicated the production of antibodies, and cellular response⁸¹. Both INO-4700 and INO-4800 utilize Inovio's CELLECTRA, which generates an electric pulse to open pores that allows the passive entry of the delivery vehicle into the cells. CELLECTRA is a small, handheld easy-to-carry device, that can be measured in large quantities in a short period⁸². These trials showed the binding site for these vaccines is angiotensin-converting enzyme 2 receptor. After a single dose of vaccine, antibodies were found in the lungs, preventing the replication

and increasing the count of the virus in the lungs⁸⁰. INO-4800 when administered to healthy adults (18 to 50 years of age) in a dose of 1 to 2 mg induces humoral and cellular immunological response in the volunteers. The second dose was administered with a gap of 1 month (4 weeks)⁸³. In addition to the intramuscular route, the oral route of administration is attractive as this route is safe with more patient compliance and the cost is less and affordable as compared to other routes^{84,85}. The other advantage of the oral route is the gastrointestinal tract having a 300 m² area, serving as an immune inductive site^{84,86}. Symvivo developed a bacTRL-Spike vaccine, comprising of gel capsule (lyophilized) of probiotic bacteria that has been genetically modified. These bacteria can colonize in the gastrointestinal tract; can bind to the intestinal membrane of the gut wall and here can secrete and can reproduce thus delivering plasmid DNA molecules encapsulating spike protein of Coronavirus⁸⁷. Another oral vaccine, a blessing for humans, preventing the pain of needle piercing is Genexine's GX-19 in phase I clinical trial⁸⁸. It is injected by a jet injector with high pressure to penetrate the skin through skin pores without the use of needles⁸⁹. This technology is found to be safe and effective in treating many viral ailments including measles and influenza. The other development companies AnGes and Zydus are developing their DNA plasmid candidates. AnGes candidate's route of administration is intramuscular and the Zydus vaccine has an intradermal route with successful clinical trials^{90,91}.

Viral vector (Oxford University/AstraZeneca):

For the development of viral vector-based COVID-19 vaccine (Covid proteins on the surface), non-replicating adenovirus, adenovirus type V, and chimpanzee adenovirus is genetically altered⁹². Adenovirus is employed for scalability and adjuvant properties. The only disadvantage of employing adenovirus is the already available immunity in humans against adenovirus⁹³. It is advantageous to use adenovirus because it doesn't replicate and thus has no chance of continuous infection and the vaccine is effective after its single dose. The only side effects that are found in vaccinated people are high body temperature, body pain, and headache.

Live attenuated viruses:

These types of vaccines can be prepared by utilizing a non-pathogenic and less virulent form of SARS-COVII virus (pathogenicity of COVID-19 virus is

weakened by genetic engineering or by genetic mutations). The advantage of using live attenuated vaccine is the availability of potent immune system stimulating but not disease-causing form of virus. But the non-pathogenic form can be altered to a pathogenic deadly form⁶⁰.

Inactivated vaccines (IVs)

These vaccines are prepared by inactivating the pathogens by chemical modification or by heat. These vaccines have lower immunogenic potency than live attenuated vaccines but are safer than LAVs⁹⁴.

Protein-based vaccines

Subunit vaccines with antigenic fragments (Novavax, phase 1/2 NCT04368988)

Glycoprotein-based nanoparticles with molecular adjuvants (Matrix M adjuvants or protein S with S1/S2 adjuvants) for the enhancement of immune response against the virus. The vaccine primarily contains the antigenic fragment of the virus, by eliminating the non-pathogenic parts not involved in the immunogenicity of the virus to reduce the side effects. Companies are also trying to develop subunit vaccines by utilizing receptor-binding domains of spike protein⁵⁸.

DEVELOPMENT OF VACCINES

Pre-clinical trials

Before the development of vaccines, before clinical trials on humans, its safety profile is determined by performing preclinical trials.

Phase I trials

In phase I trials, vaccines are tested on a small population (20-50) by comparing with inactive substance (placebo) to determine the vaccine's safety, dose, minimal interval required between vaccine doses and adverse effects.

Phase II trials

After Phase I trials, if the vaccine is declared safe, it is tested on a large population (200 volunteers). At this stage, a vaccine is tested to determine its immunogenic response (either the vaccine can produce an immunogenic response in the recipient or the time required to produce this response) and the interval between dose's administration in detail.

Phase III trials

Once, phase II results satisfy the clinicians, vaccines are tested on a large scale (3000 – 50,000) people. These trials can take 5 years, and measure the effectiveness of the vaccine in the large pool. After this stage, the vaccine needs to be approved by regulatory bodies, the Food and Drug Administration (FDA) and EMA (European Medicines Agency) for large-scale production.

Phase IV trials

After the distribution of vaccines to the market, and its administration to the public, it becomes part of pharmacovigilance. At this stage, the drug is tested for its adverse effects, causes of AE, prevention and other parameters of immunization⁹⁵.

PRODUCED VACCINES

Pfizer/BNT162b2/BIONTECH

On December 2, the United Kingdom gave emergency authorization to Pfizer vaccine for administration. In the same month, on 31st December 2020, WHO approved it on an emergency basis making it the first approved vaccine against Coronavirus. Pfizer is a lipid-based nano-particulate RNA vaccine that contains COVID-19 virus-stabilized spike protein. After the penetration into the cells, the vaccine produces the virus's spike proteins as antigens for the production of antibodies and the activation of immune response⁹¹.

In a study conducted, 43,448 patients were selected, 50% of the patients were given vaccine shots and the remaining half were given placebo. After the administration of the second dose of the Pfizer vaccine, 8 out of 21720 patients got COVID-19, in the patients receiving a placebo 162/21728 were affected by covid. The data suggested 95 % efficacy of the Pfizer vaccine. The vaccine was equally effective with a 95% confidence interval in different subgroups, people of different ages, genders, ethnic groups and different body mass index. This trial was sponsored by BioNTech, and Pfizer conducted the trials and collected and interpreted the data from the people. The usual dose of Pfizer is two doses of 30 µg administered intramuscularly at a minimum interval of 3 weeks. The introduction of the Pfizer vaccine triggers neutralizing antibody production and specific CD8+ and CD4+ cell response. BNT162b2 was found effective in confirmed cases of COVID-

19 having symptoms like cough, pyrexia, difficulty in breathing, muscle pain, diarrhea, vomiting, and loss of taste and smell. A phase II clinical trial was conducted including 44820 persons including ethnic groups, gender and race from all over the world. The persons receiving the BNT162b2 vaccine showed a median safety profile throughout the trial. Two persons receiving the vaccine have died, but the deaths were associated with heart disease not because of the vaccine. A male worker who received the Pfizer vaccine with no previous medical history got a severe headache, hyperthermia, and pruritus after the first dose and measles-like eruptions after the second dose ⁹⁶.

Moderna

Moderna is mRNA-based vaccine like the Pfizer. It can be stored at -20°C and thus has an advantage over Pfizer, which needs -75°C temperature. Moderna can be stored in a conventional freezer for up to six months and after thawing can be kept for one month in a standard refrigerator. This feature makes it easier to use as compared to DNA vaccines. It was first approved in December 2020 by the FDA against COVID-19 pandemic. The vaccine is safe in humans and has minor adverse effects, in the phase III clinical trial named COVE, just 1.5 % of the individuals showed hypersensitivity, fatigue and pain at the injection site ⁹⁷. Anaphylactic cases were found to be 5.5 per million after vaccine administration. These mild symptoms disappear after a few days of administration of a vaccine. However, four severe cases of rashes and morbilliform rashes were observed in this trial. In this trial, Moderna's developed mRNA-1273 trial COVE, out of 30,420 individuals, 50% of the population received a placebo while half received the vaccine. The results showed that 185 participants of the trial got symptomatic COVID-19, while just 11 participants who received Moderna's mRNA vaccine got the disease showing 94% efficacy of the vaccine. The vaccine's efficacy was observed to be less in elder people at 86.4% as compared to 96% efficacy in the young group. The participants showed maximum efficacy against vaccine approx. After 10 days of dose administration. This trial included 30,000 United States citizens, with almost 7000 above the age of 65 years, 5000 below the age of 65 years, 3000 black US citizens and 6000 Hispanic.

After mass administration of Moderna in the general public and patients were experiencing the pruritic

patches one week after the dose administration ⁹⁷. The results revealed that there was no relation between medical history and the development of rashes, the problem was revealed in some individuals after dermatological treatment, while in some resolved spontaneously. Moderna and Pfizer used the same ingredients, but the rashes appeared only with the Moderna vaccine.

The general public is somehow resistant to vaccination. In a study conducted by the Kaiser Family Foundation on US adults, one-third of the survey participants showed hesitancy by giving the statement, that they will not get vaccinated even if it is free and safe. About, 60% population was reluctant and worried because of possible adverse effects of vaccine ⁹⁸.

VACCINE DOSAGE REGIMEN

14 days after the administration of the second dose, Moderna showed 95% efficacy against Coronavirus ⁹⁹. Regulatory Agency of Medicines and Healthcare Products (MHRA) approved doses of Moderna with a gap of 28 days between the two doses ¹⁰⁰. According to the Disease Control and Prevention Center (CDC), there should be a gap of 3 weeks between the doses of Pfizer, and 4 weeks for Moderna. There should not be a gap of more than 6 weeks between the doses of both Pfizer and Moderna, but if the dose is not given within a specific time, it can be given without repeating the first dose. In case of emergency, a second dose can be given 4 days before the specified period. Pfizer is recommended for people 16 years of age at a dose of 30 μg , while Moderna can be given to people above 18 years of age at a 50 μg dose.

Why are 2 vaccine doses needed?

Clinical trials revealed that after the first dose, the immunity provided by the vaccine was weak, which necessitates the administration of a second dose. The second dose induces a strong immune response in the body. All the trials were conducted with the two doses of vaccine; no trial has been conducted with one dose only. It is assumed that one dose will provide a partial immune response against the virus.

Effect of variants on vaccine's efficacy

New variants have increased the spread of the virus. The studies showed that approved vaccines work against the new variants. The basic mechanism behind the vaccine's development is to target

different parts of the virus, so if in one variant, one part mutates, the vaccine can act on the other part to start antibody production. New variants can reduce

the efficacy of a vaccine but cannot nullify the effectiveness¹⁰¹.

Table 3. List of COVID-19 vaccines and clinical trials

Vaccine type	Developer	Antigen used	Vaccine	Delivery system utilized	Route	Clinical Trial
Virus-like particles	Medicago	VLP from virus spike protein	CoVLP	Recombinant Coronavirus Virus-Like Particle (CoVLP)	IM	NCT04450004
mRNA	Moderna BioNTech Pfizer	Receptor binding domain of Coronavirus Complete length of the spike protein	mRNA-1273 BNT-162b2 BNT-162a1 BNT-162b1 BNT-162c2	Lipid nanoparticle	IM	NCT04470427 NCT04368728
DNA	Osaka University Inovio Pharmaceuticals International Vaccine Institute	Spike protein	AG0301-COVID19 INO-4800	Plasmid	IM ID	NCT04463472 NCT04527081 NCT04470427
Recombinant protein	University of Queensland Novavax	Spike protein perfusion state	UQ-CSL V451 NVX-CoV2373	Spontaneous nanoparticle formation	IM	ISRCTN51232965 NCT04368988

CONCLUSION

The current global condition induced by COVID-19 requires the urgent need for effective vaccine development and their safety profile. COVID-19 is not novel and viruses from the same family have been causing the disease in humans for decades, thus exposure of humans to the same virus type has made it easy for scientists to develop vaccines in the shortest period with better safety profiles. Along with safety and effectiveness, physicochemical properties of vaccines, storage conditions, transport issues, and cost balancing are the main concerns to make vaccines available for countries all over the globe to make the world a safe place to live and COVID-free. Vaccination is the right of each country, irrespective of its economic status. RNA vaccines fulfill all these requirements, these are safe and easy to produce in a short time and at an affordable cost. However, the effectiveness of these clinically approved vaccines in humans is less as compared to the animal model and has some serious and unpredictable side effects in some humans even the humans don't have a previous

history of any medical condition. The changing nature of COVID-19 is another challenge to the researcher. New variants with altered genomic sequences have evolved which not only affects the efficacy of developed vaccines but also alarms that scientists should develop effective counter measures against emerging new pathogens. This review has enlightened novel variants of COVID-19 and highlighted the mutations and possible structural target points. This article will serve as a hallmark for understanding virus mutation mechanisms and strategies to combat these mutation points.

Author Contributions: Concept/Design : UI, SSJ, TA, RA, AK, AA, YR, SMM, ZK; Data acquisition : UI, SSJ, TA, RA, AK, AA, YR, SMM, ZK; Data analysis and interpretation : UI, SSJ, TA, RA, AK, AA, YR, SMM, ZK; Drafting manuscript : UI, SSJ, TA, RA, AK, AA, YR, SMM, ZK; Critical revision of manuscript : UI, SSJ, TA, RA, AK, AA, YR, SMM, ZK; Final approval and accountability : UI, SSJ, TA, RA, AK, AA, YR, SMM, ZK; Technical or material support : UI, SSJ, TA, RA, AK, AA, YR, SMM, ZK; Supervision : UI, SSJ, TA, RA, AK, AA, YR, SMM, ZK; Securing funding (if available): n/a.

Ethical Approval: As this research is narrative review, ethics committee approval is not required.

Peer-review: Externally peer-reviewed.

Conflict of Interest: Authors declared no conflict of interest.

Financial Disclosure: Authors declared no financial support

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