A review of SARS-CoV-2 virology, vaccines, variants and their impact on the COVID-19 pandemic

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A novel coronavirus, named severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2) has spread in Wuhan, China, and caused the global pandemic infectious disease. This disease has been known as coronavirus disease 2019 (COVID-19). It continued to spread around the world and created outrageous effects on the healthcare and economic system throughout the world. Various strategies have been designed to diminish the morbidity and mortality of this infectious disease. Among them, the development of vaccines is the most effective method to prevent and treat the viral infection. Novel vaccines have been developed and proved to be effective in multiple clinical reports indicating a significant decline in the risk of COVID-19 infection. However, the emergence of new variants of SARS-CoV-2 with immune-evasive characteristics raised questions concerning the effectiveness of the vaccines. This review provides a brief introduction to developed vaccines, as well as emerging variant strains and vaccine effectiveness against these variants. In this article, we also reviewed the general biological features of SARS-CoV-2 and its pathogenesis and explained the clinical symptoms, transmission, diagnostic and treatment approaches to monitor and control emerging COVID-19 infection.

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Introduction

The outbreak of pneumonia in December 2019 occurred in China's Hubei province, raising global health concerns due to its rapid transmission to other areas. After research on numerous cases, a novel coronavirus was identified as the cause of the disease and, On February 11 of the following year, that causative pathogen was named by the institutional Virus Classification Commission as severe acute respiratory syndrome-coronavirus-2 (SAR-CoV-2) due to its high homology (∼80%) to SARS-CoV (which caused acute respiratory syndrome during 2002–2003) [1] and the resulting disease was named by the World Health Organization (WHO) as coronavirus disease 2019 (COVID-19) [2,3]. As infection has spread globally with deadly effects, the World Health Organization declared COVID-19 a global pandemic on March 11; 2020 [4,5]. The first case of the novel coronavirus (SARS-CoV-2) was released in December 2019. Since then, this virus has continuously caused global health calamities and caused drastic impacts in all fields including; environmental, social, political, cultural, and economic. The most effective method to reduce the drastic effects of viral infectious disease is vaccination. Several vaccines have already been developed and marketed worldwide at unprecedented speed and scale, while a number of vaccines are in the process of being developed and more than 200 different candidate vaccines are in different stages of development [6]. As of 2 December 2022, 11 different vaccines have been granted Emergency Use Listing (EUL) by the WHO. These include two mRNA vaccines (Pfizer–BioNTech-Comirnaty and Moderna-

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Spikevax), two protein subunits vaccines (Novavax and COVOVAX), four adenoviral vector vaccines (CanSino-Convidecia, Johnson & Johnson, Oxford–AstraZeneca, and COVISHIELD), and 3 inactivated vaccines (Sinopharm, Sinovac, and COVAXIN) [7]. Globally, as of 21 September 2023, there have been 770 778 396 confirmed cases of COVID-19, including 6958 499 deaths, reported to WHO. 70.5% of the world population has received at least one dose of a COVID-19 vaccine. As of 18 September 2023, a total of 13 505 089 801 vaccine doses have been administered. 20 739 are now administered each day. 32.6% of people in low-income countries have received at least one dose [8]. In this review, we discussed developed vaccines and the impacts of variants on the effectiveness of these vaccines as well as challenges regarding the development of vaccines and the vaccination process to curb this pandemic. In this report, we also present the clinical work performed in our laboratory (Hirahata Gene Therapy Laboratory, HIC-Clinic) regarding the treatment of this infection and review it with the current research.

Etiology of coronavirus

A human coronavirus was isolated for the first time from the nasal secretions of a male child with a common cold in 1965 [9]. Coronaviruses (CoVs) are nonsegmented, enveloped, single-strand, positive-sense RNA viruses ranging from 60 to 140 nm in diameter with spike-like projections on their surface giving them a crown-like appearance therefore, coronaviruses (CoVs) are named due to their Sun's corona or crown-like surface projections [10,11]. CoVs are the largest group of host-specific RNA viruses infecting birds, snakes, bats, and mammals including humans. They belong to the family Coronaviridae (order Nidovirales), which is divided into subfamilies Coronavirinae and Torovirinae. The subfamily Coronavirinae is further divided into four genera: Alphacoronavirus (alphaCoV), Betacoronavirus (BetaCoV), Gammacoronavirus (GammaCoV), and Deltacoronavirus (deltaCoV) [12,13]. CoVs grouped into Alphacoronaviruses and Betacoronaviruses, which are mostly found in mammals like bats, civets, rodents, and humans while Gammacoronaviruses and Deltacoronaviruses are mostly found in birds. It is reported that beta-coronaviruses cause severe disease while alpha-coronaviruses cause asymptomatic or mildly symptomatic disease [14]. To date, seven human CoVs (HCoVs) capable of infecting humans have been identified including HCoV229E, HCoV-OC43, HCoV-NL63, HKU1, SARS-CoV, MERS-CoV and SARS-CoV-2 [15,16]. Some of the HCoVs were identified in the mid-1960s, while others were only detected in the 21st century. In general, estimates suggest that 2% of the population is healthy carriers of CoVs and that these viruses are responsible for about 5% to 10% of acute respiratory infections [17]. Among seven human CoVs (HCoVs), two betaCoV (HCoV-229E and HCoV-HKU1) and two alphaCoV (HCoV-NL63 and HCoV-OC43) genera have been circulating in human and developed upper respiratory disease [18,19]. Three coronaviruses (beta coronavirus/ betaCoV genus) that cause serious respiratory disease are SARS-CoV, MERS-CoV, and SARS-CoV-2 which are responsible for high mortality rates in humans. In the 21st century, three CoVs named SARS-CoV, MERS-CoV, and SARS-CoV-2 outbreaks emerged from their reservoirs to cause serious respiratory disease in humans. Among them, severe acute respiratory syndrome coronavirus (SARS-CoV) emerged in late 2002, and Middle East respiratory syndrome coronavirus (MERS-CoV), emerged in 2012, which have been previously implicated in epidemics with mortality rates up to 10% and 35% respectively, [20,21] and SARS-CoV-2 emerged in 2019 in China as a threat to the human lives and caused COVID-19 disease. SARS-CoV-2 has become a major public health concern after the outbreak of the MERS-CoV and SARS-CoV [15,16,20].

Role of spike protein in the pathogenesis of SARS-CoV-2 infection

Phylogenic analysis revealed that SARS-CoV-2 is closely related to the beta-coronaviruses. Similar to other coronaviruses, the genome of SARS-CoV-2 is positive-sense single-stranded RNA [(+) ssRNA] with a cap at the 5′end, and a polyadenylated (polyA) tail at the 3 end' [22]. The genome of SARS-CoV-2 has a length of about 30K nucleotides. It has other 5–8 accessory proteins (ORF3a, ORF6, ORF8, ORF7, and ORF9), nonstructural proteins (NSPs), structural proteins, and accessory proteins. Nonstructural proteins such as RNA polymerase, RdRp; papain-like protease, PLpro; coronavirus main protease, 3CLpro) are encoded by the ORF region [21]. Nonstructural proteins (NSPs) such as (NSP3, NSP9, NSP10, NSP12, NSP13, NSP15, and NSP16) play important roles in viral replication, cleavage of host mRNA, membrane rearrangement, assembly processes, capping, tailing, and methylation, etc. which are vital for viral life cycle and pathogenesis [23–25]. Four structural proteins of the SARS-CoV-2 virus named S-spike Protein (outer spiky glycoprotein), envelope protein (E), Membrane/matrix glycoprotein (M), and nucleocapsid protein (N) share high sequence similarity to those from SARS-CoV and MERS-CoV [20,24,25]. These proteins help the coronavirus-host cell interactions to create an optimal condition for viral entry in host cells, viral replication, and induction of the immune system by eliciting neutralizing antibodies. Thus, coronavirus-host interaction plays a vital role in the viral disease pathogenesis [26]. Amongst the four structural proteins,

the S protein (a transmembrane glycoprotein) that covers the surface of the SARS-CoV-2 virus, plays the vital role of virus binding to the receptor (angiotensin-converting enzyme 2-ACE2), which is expressed on the host cell, antigenic recognition, viral fusion, and virus entry into the host cell [23,27]. The S protein has two subunits S1 and S2. The subunit S1 is responsible for the binding of viral envelops to the host cell receptor, while the S2 subunit contains a fusion peptide, a transmembrane domain, and a cytoplasmic domain that facilitates the fusion of the virus-cell membrane to the host cell [28,29]. Further, the S1 subunit is split into different functional domains, receptor binding domain (RBD) and N-terminal domain (NTD) which facilitates viral entry into the host cell and serves as a potential target for neutralization in response to vaccines [30]. Structural and functional analysis showed that the RBD of the S1-subunit (SARS-CoV-2 spike or S protein) is a fundamental peptide domain in the pathogenesis of infection as it represents a binding site for the human angiotensin-converting enzyme 2 (ACE2) receptors [31–33]. Following the binding of the S protein to the (ACE2) receptor on the host cell surface, the spike protein activated via undergoing protease cleavage by enzyme trans-membrane protease serine 2 'TMPRSS2', which is located on the host cell membrane and promotes virus entry into the cell by activating the S protein. Once the virus enters the cell, the viral genome is released into the host cell, replication and transcription of the viral RNA is occurred, structural protein is synthesized and assembled in the host cell, then viral particles are released from host cells by the exocytic pathway [34,35].

Symptoms of SARS-CoV-2 infection

SARS-Cov-2 virus primarily affects the respiratory system although other organ systems such as the lung, brain, kidneys, immune system, and heart are affected directly or indirectly [36]. Acute COVID-19 infection symptoms vary from asymptomatic/mild symptoms to critical illness with acute respiratory disease and death. Most common symptoms are very similar to seasonal flu such as fatigue, headache, cough, fever, shortness of breath, and other symptoms reported in acute conditions include vomiting, muscle pain, diarrhea, myalgia, sputum production, dyspnea, and hemoptysis. Patients with serious symptoms such as difficulty breathing, chest pain, and loss of speech or movement need urgent medical attention [37–40].

Clinical diagnosis of SARS-CoV-2 infection

Reliable and early diagnosis of infection plays a pivotal role in combating the pandemic COVID-19. A reliable and specific diagnostic test is the most effective approach to identify the infected patients in the mass population during this pandemic infection. Three types of diagnostic tests are available to identify the infection; molecular or nucleic acid amplification tests (e.g., PCR tests) that detect viral RNA; antigen tests that detect viral proteins (e.g., nucleocapsid or spike proteins); and serology-based tests that detect viral antigen or host antibodies in tissues, secretions, or eliminations from individuals with ongoing or past infections [41]. The use of the quantitative reverse-transcription polymerase chain reaction (RT-PCR) assays for the detection of viral nucleic acid is the most valuable assay in terms of its specificity, reliability, high capacity, and speed. It has been shown to be very sensitive for accurately detecting the viral genome, and able to detect a single copy of the viral RNA [42]. Three highly conserved regions (RdRp, E, and N gene) have been found in the SARS-CoV-2 viral genome, and are usually selected as the reliable targets for the design of primers and probes [43]. It is essential to update the sequences of primers and probes to ensure the detection of newly emerged variants.

A novel antigen-based rapid test for diagnosis of SARS-CoV2 showed high sensitivity and specificity mainly in the first week among symptomatic patients and samples with high viral load [44].

Serological assays such as enzyme-linked immunosorbent assay (ELISA), chemiluminescent immunoassay (CLIA), and immunofluorescent assay (IFA) are based on targeting S protein and N protein antigens for the rapid detection of SARS-CoV-2 through measure immunoglobulin M (IgM) and/or immunoglobulin G (IgG) antibodies in serum or body fluid samples [45].

All three types of tests are available as laboratory-based assays that can be performed by lay health-care providers outside of laboratory settings and with minimal training. Although sensitivity and specificity are important attributes of a diagnostic test, they differ between populations and the types of equipment employed [46]. A correct diagnosis in a patient also depends on the time of sampling relative to the stage of infection, the quality of the specimen collection, proficiency to perform a test, and correct interpretation of the results.

Transmission of SARS-CoV-2 virus

Animal-to-human transmission

The source of origination and transmission are essential to be determined in order to prevent transmission of disease. In the case of SARS-CoV, initially, researchers found palm civets as a key reservoir of infection. The samples isolated from the civets at the food market showed positive results for viral RNA detection [47]. Further,

wide-reaching investigations of farmed and wild-caught civets revealed that the SARS-CoV strains found in market civets were transmitted to them by other animals [48] suggesting that the palm civet might be the secondary host of infection. Another report revealed the discovery of novel coronaviruses related to human SARS-CoV, which were named SARS-CoV-related viruses or SARS-like coronaviruses, in horseshoe bats (genus *Rhinolophus*) [49,50]. These discoveries also suggested that civets were only intermediate hosts and bats may be the original host for SARS-CoV. Due to similarities between SARS-CoV and SARS-CoV-2, a group of scientists and researchers believed that like SARS-CoV, another animal might be present as an intermediate host to transmit SARS-CoV-2 to humans. Therefore, finding the intermediate host is pivotal to developing preventive strategies to stop interspecies transmission of SARS-CoV-2. In this regard, some studies also revealed that SARS-CoV2 have been transmitted to humans through other intermediate animals potentially sourced from the Huanan wholesale seafood market which sells bats, snake, and other poultry in Wuhan City, Hubei province, China [51–53]. Furthermore, phylogenetic analysis of the SARS-CoV-2 genome indicates that the virus is closely related (with 88% identity) to two bat-derived SARS-like coronaviruses collected in 2018 in eastern China (bat-SL-CoVZC45 and bat-SL-CoVZXC21). Moreover, after genomic similarity findings of novel coronavirus with SARS-like bat viruses, some researchers suggested that only bats could be the key reservoirs of this infection [51,54]. Later on, further study found that the virus is more related to BatCoV RaTG13, a bat coronavirus detected in *Rhinolophus* affinis bat which has 96.2% whole-genome sequence similarity [55]. Another study based on relative synonymous codon usage (RSCU) on a variety of animal species also revealed that bats are the most probable wildlife reservoir of SARS-CoV-2 [56]. Altogether, these findings suggest that bats might be the original host of this virus [57]. However, bat-derived coronavirus (CoVs) rarely infect humans therefore an intermediate host must be there for transmission of SARS-CoV-2 to humans. Therefore, further research was performed to find out that intermediate host, and according to one study, pangolins have been suggested as an intermediate host for SARS-CoV-2, Malayan wild pangolins were tested for SARSCoV-2-like coronaviruses, with major amino acid identity with SARS-CoV-2 in the E, M, N and S proteins, respectively. In particular, the receptor-binding domain (RBD) of the spike protein of the Pangolin-CoV was found to have a minor difference in only one amino acid from that of SARS-CoV-2. Comparative genomic analysis further indicated that the SARS-CoV-2 may have potentially originated from the viral recombination to Pangolin-CoV with Bat-nCoV (RaTG13) before transmitting to humans [58]. Nonetheless, finding the intermediate SARS-CoV-2 host is pivotal to developing preventive strategies to stop interspecies transmission, and more research work is required to perform in the aspects of the identification of the intermediate source that caused the transmission of the virus to humans.

Human-to-human transmission

The principal mode by which people are infected with SARS-CoV-2 is through exposure to respiratory fluids carrying infectious viruses. Infectious exposures occur in three different ways (1). Direct inhalation of air carrying very fine respiratory droplets and aerosol particles released into the environment by sneezing or coughing. (2) Deposition of respiratory droplets and particles on exposure with oral, nose, or eye mucous membranes. (3) Touching mucous membranes with hands that have been soiled either directly by respiratory fluids containing the virus or indirectly by touching inanimate surfaces contaminated with the virus. Therefore, COVID-19 can be transmitted through direct contact with infected people or indirectly, through the surface contaminated by infected person [59–63].

Variants of SARS-CoV-2 virus

Like other RNA viruses, the SARS-Cov2 virus is unstable and prone to genetic evaluation caused by genetic mutations or viral recombination resulting in the development of variants that have different characteristics compared to its ancestral strain (the original SARS-CoV-2 virus). Throughout the COVID-19 pandemic, many variants of SARS-CoV-2 have been found globally. Though coronaviruses such as SARS-CoV-2 are relatively stable as they possess a genetic proofreading mechanism due to the presence of a nonstructural protein (nsp) with 3′-5′ exoribonuclease activity (nsp14) [64]. Therefore, the rate of variant accumulation is slower than for other RNA viruses such as HIV-1 or influenza A. However, Korber *et al.*, with the analysis of accumulating GISAID sequence data developed a bioinformatics approach identifying specific viral variants carrying the D614G mutation in the Spike protein of SARS-CoV2 which rapidly became the dominant widespread variant of SARS-CoV2 across the world [65]. Another variant was identified in humans, attributed to transmission from infected farmed mink in Denmark [66]. Since then, many genomic studies have revealed changes in the genome of SARS-CoV-2, and multiple variants have been defined by diffident lineage based on the sequences of the spike protein. However, the mutation also occurs in regions beyond spike proteins that contribute to the variant's distinct features [67]. These viral variants cause an increase in the efficiency of viral transmission, enhancing viral replication, disturbing viral fitness, cell tropism, disease severity, drug resistance, pathogenicity, and escape immune recognition [68].

With the continued emergence of multiple variants, in late 2020, the WHO defined variants as variants of interest (VOIs), variants of concern (VOCs), and variants under monitoring (VUMs) in the function of their genome mutations, their properties of spreading between susceptible hosts, the disease severity produced, and by the evasion of the immune response elicited by currently available vaccines. Among them, two major types of viral variants, VOIs and VOCs are identified to fight against the pandemic [69].

Alpha (B.1.1.7 or 20I/501Y.V1)/UK variant: The B.1.1.7 variant, the first variant of concern, is known as the Alpha variant or GRY. It was first reported in the southeast of the United Kingdom in September 2020 and became the dominant variant and circulated worldwide and designated as a VOC and labeled Alpha by the WHO on December 18, 2020. (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/) [70,71]. This variant is characterized by the presence of approx. 17 mutations in the viral genome. Most of them including (H69-V70 deletion, Y144 deletion, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H) are found in the spike protein as compared with the original virus isolated in China [72]. Among the mutations, N501Y's protruding protein mutation plays an extremely important role in enhancing the binding affinity of S protein with hACE-2 receptors and enhancing the ability to enter host cells [73–75]. Compared with other VOCs, it has a 50% higher transmissibility [76]. According to one trial report, AstraZeneca's vaccine has been shown to be 70% effective against alpha VOCs [77]. Another trial study reported that the Pfizer-BioNTech (BNT162b2) vaccine was 89.5% effective against the Alpha variant after receiving two doses [78].

Beta (B.1.351 or 20H/501Y.V2)/South Africa variant: B.1.351, also known as 501Y.V2 was first detected in South Africa in May 2020 after the first epidemic wave in Nelson Mandela Bay and spread rapidly in Eastern Cape, Western Cape, and KwaZulu-Natal provinces in just a few weeks, causing the second wave of epidemic in South Africa [79]. It was designed as a VOC and labeled Beta by the WHO on December 18, 2020 (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/). This variant contains nine mutations (L242_L244 deletion, D80A, D215G, K417N, E484K, N501Y, D614G, and A701V) in the spike protein as compared with the original SARS-CoV-2 virus [72]. Of which 3 mutations (K417N, E484K, and N501Y) are located in the receptor binding domain (RBD), increasing the binding affinity with hACE-2 receptors, resulting in a higher risk of transmission [80]. The mutation E484K in this variant mediates the antibody escape is the main reason for the reduced sensitivity to vaccines [81]. According to the findings of a trial, the Pfizer-BioNTech vaccine has been shown to be 75% effective against this viral variant after two doses [78]. And Novavax vaccine showed 86% efficacy against Beta variants in the UK and 60% in South Africa, respectively [82], the trial results for the ChAdOx1 nCoV-19 vaccine from Oxford-AstraZeneca revealed low efficacy (10%) after two doses against this variant [83].

Gamma (P.1 or 20J/501Y.V3)/Brazilian variant: the P.1 variant, also known as the Gamma variant or GR/501Y. V3. It was primarily found in North Brazil in the city of Manaus in the Amazonas state in December 2020 and caused the second wave of the epidemic in this country [84]. By January 2021, it was identified at the National Institute of Infectious Diseases (NIID) in Japan during analysis of samples of four travelers/passengers from Brazil and was designated as VOC and labeled Gamma by the WHO on January 11, 2021. (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/). This variant arose from lineage B.1.1.28 and has 12 mutations in its spike protein (L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, and V1176F) [72]. L18F, K417N/T, E484K, N501Y, and D614G are located in the RBD, similar to the B.1.351 variant [84,85]. These mutations have been shown to have important implications, both in the transmissibility of the virus and the rate of reinfection [86]. When compared to the other prevalent variants in the country, it was found 1.7–2.4 times more transmissible [82] In another report, it was observed that this variant is 2.2 times higher transmissible and led to a few cases of reinfection recovered from COVID-19 [87,88]. According to one study, ~50% efficacy of two doses of Pfizer or Oxford-AstraZeneca vaccines against this variant was observed [89]. In the case of CoronaVac, the vaccine showed 37–59% efficacy against this variant [90,91]. In contrast, Moderna (mRNA-1273/Moderna) showed a reduced efficacy of protection (61%) after two doses [92]. Another clinical trial demonstrated that the CoronoVac vaccine is 50% effective against the Gamma variant in Brazil [93].

Delta (B.1.617.2)/India variant: B.1.617.2, also referred to as the Delta variant or GK was first detected in Maharashtra, India in October 2020, causing a second wave of deadly COVID-19 infections in India [69]. This variant was designated as VOC and labeled Delta by the WHO on May 11, 2021, (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/). VOC Delta was a worldwide circulating variant after the Alpha variant and was reported by at least 169 countries (https://outbreak.info). In comparison to other VOCs, this variant has had deadly global impacts [94]. This variant is characterized as highly transmissible and has spread worldwide between fully vaccinated as well as in unvaccinated individuals [95]. According to reports from UK studies, this variant is 60% more transmissible compared to the Alpha variant [82]. There were ten mutations in the S protein of Delta variant: T19R, T95I, G142D, E156G, Del157/158, L452R, T478K, D614G,

P681R, D950N (https://outbreak.info) [96]. The current vaccines on the market have shown to be less efficient against Delta VOCs than the Alpha variant. The Pfizer vaccine has been shown to be 88% and 93% effective against the Delta and Alpha VOCs, respectively, after a full dose. The AstraZeneca vaccine has been shown to be 60% and 66% effective against Delta and Alpha strains after two doses, respectively [82]. The mutations like L452R, and T478K have not been reported in previous VOC Alpha, Beta, and Gamma, these mutations gave VOC Delta a stronger transmission ability and immune escape ability, which made the Delta variant quickly become a dominant variant and reduced the efficacy of approved vaccines. The emergence of this variant despite widespread vaccination raised questions about the efficacy of current SAR-CoV-2 vaccines (https://aci.health.nsw.gov.au/covid-19/critical-intelligence-unit/sars-cov-2-variants).

Omicron (B.1.1.529): first reported in South Africa in November 2021

This variant (B.1.1.529 lineage) was identified on November 11, 2021 in Botswana, Africa, and was designated as VOCs and labeled Omicron by the WHO on November 26, 2021 (https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/). Omicron consists of four genetically related viral sub-lineages (BA.1, BA.1.1, BA.2, and BA.3) that diverged from the B.1.1.529 lineage [97]. It has more than 30 mutations, including T95I, K417N, N501Y, T478K, N679K, G142D/143−145del, and P681H. These mutations are already reported in previous VOC (alpha, beta, gamma, and delta) [98]. Six of these mutations (G339D, N440K, S477N, T478K, Q498R, and N501Y) enhance the binding affinity to the human hACE-2 receptor, while seven mutations (K417N, G446S, E484A, Q493R, G496S, Q498R, and N501Y) are associated with a reduction in neutralization [99]. Omicron has mutation such as N501Y, which is present in the B.1.1.7, B.1.351, P.1, and P.3 lineages, and associated with improved binding of the RBD domain of the spike protein to the ACE2 receptor, resulting in higher transmissibility [100,101]. It also shares the K417N and E484K mutations (E484A in the Omicron variant) with the B.1.351 and P.1 lineages, which are linked to evasion of the immune response [102]. One study demonstrated that the Omicron variant is more contagious as the reproductive number of the Omicron variant is higher than that of the Delta variant [103,100]. However, the results of recent hospitalizations from the United States found that the Omicron variant was associated with less severe disease compared to Delta [104,105]. In addition, Omicron is characterized by its potential to evade the humoral immune response in individuals who have a full vaccination scheme (including the booster dose) [106,107] and therefore, Omicron is considered more infectious (2.7−3.7 times higher) than the Delta variant [108]. As mentioned, Omicron has so many common mutations described in other VOCs, that it is expected that available vaccines may help control the further prevalence of SARS-CoV-2 cases, but still more research is required in this regard. According to one study, the efficacy of the Pfizer vaccine against this variant decreases 28.2-fold, however, a third dose increases the effectiveness of the Pfizer vaccine 23.4-fold [106,107], suggesting that the third boost of the Pfizer vaccine can efficiently neutralize this variant. As well as the combination of the Johnson and Johnson (Ad26.COV2) vaccine with a booster of Pfizer, also crucially increases the activity of neutralizing antibodies against Omicron [107]. Omicron-associated lineages, such as BA.1, BA.1.1, BA.2, BA.3, and XE (BA.1 and BA.2 recombinant), are still emerging. Moreover, new variants such as the Deltacron a new recombinant variant that originated from the recombination in the Delta and Omicron coinfected patients have appeared in many countries like France and America, although the detected sequence of Deltacron was lower than Omicron [109,110].

Conclusively, the emergence of new variants in the SARA-CoV-2 genome has become one of the primary challenges facing coronavirus disease because a genetic mutation in the SARS-CoV-2 genome could alter its pathogenic potential, and would increase the difficulty in the diagnosis and treatment of infection and also create trouble in drug and vaccine development against the coronavirus [111,112]. The investigation of the variants of SARS-CoV-2 has suggested that the coronavirus can evolve with high resistance to immune system neutralization. There is variability observed among different individuals as a response to S-protein. Therefore, a thorough study is required to investigate the efficacy of antibody neutralization in the licensed vaccines against SARS-CoV-2 variants [113,114]. New Variants of SARS-CoV-2 not only cause increased transmissibility, morbidity, and mortality rate but also can escape from detection by currently available diagnostic tests, which can potentially delay the diagnosis and treatment, therefore encouraging the need for improvement in the form of more adaptive diagnostic techniques for detection of COVID-19 infection [115].

Three waves of SARS-CoV-2 infection

People from different countries all over the world have faced three waves of COVID-19 infection to date. These waves of SARS-CoV-2 are driven by its variants which continuously set aside the accomplishments achieved through developed efficacious vaccines, social restriction, tests, different plans, and quarantine policies. These

variants result from mutations in the SARS-CoV-2 genome has introduces auger ably extreme challenges in controlling the threat of COVID-19.

First wave

The first case of COVID-19 in China was reported in December 2019 and within a week; it had spread throughout the entire world. However, first wave of COVID-19 was detected internationally in March 2020 (spring) and affected almost every corner of the world, due to atmosphere changes the southern hemisphere was affected later [116,117]. Interestingly, except in South Africa, cases and death rates with lower numbers were reported in Africa. The reason behind this may be the short average age and Ebola disease experience years ago that helped to minimize the effect of COVID-19 [118]. The 1st wave is considered a global catastrophe that drastically impacts every aspect of life including health, social life, and economy [119,120]. The economies of different societies were significantly affected globally, resulting in a sharp decline in the production of various items; consequently, unemployment rates and other social problems (violence in the family) were increased. The first wave affected multiple nations all over the world, and several experts predicted that the second wave might be stronger than the first wave [117].

Second wave

The second wave occurred at the end of summer and autumn in July 2020. Some parts of society thought that the pandemic was over and disregarded the initial announcement of the incoming second wave due to the minimum number of infection cases reported in the summer months [116]. Like other viral infections such as influenza blaze up seasonally, COVID-19 returned in autumn and the second wave hit many countries with a much higher force than the first one as predicted by multiple experts. More infection cases were reported increased number of patients in ICUs and in some countries, more deaths were recorded during the second wave [116,121,122]. During the second wave, the politician in many countries changed their priorities after the first wave faded away. In most countries, particularly in Germany, the instructions were very sharp in the 1st wave of this pandemic. Now in the second wave, the economy of society has become the first priority on the list, businesses remained open rather than strictly decreasing contacts thus infections and death rates have risen [116]. The second wave of SARs-CoV-2 showed more potential threats than the first wave and affected India very severely, as well as other countries such as the United Kingdom, Brazil, and France [123]. According to one report, the main cause of the second wave in Europe was not the relaxation of interventions during the summer, but rather the failure to enforce interventions in the fall [124]. Moreover, experts gave predictions about the third wave that would be more deadly than previous waves as the virus emerged with a new mutation.

Third wave

A third wave of COVID-19 occurred after Christmas 2020 worldwide and differential features to previous waves were observed, Such as a large proportion of cases were linked to household contacts, most likely because Christmas gatherings were the major trigger of this new wave [125]. The proportion of clinically severe cases of SARS-CoV-2 infection and mortality rates were low as compared to prior COVID-19 waves because diagnoses of asymptomatic or mildly symptomatic individuals have increased over time. Therefore, rapid antigen test availability to prompt diagnosis and then isolation [126], better clinical monitoring, and treatment of COVID-19 patients play crucial parts in controlling the spread of the third wave [127,128]. Another interesting feature in the third wave was the more transmissible B.1.1.7 strain was observed; that have higher transmissibility; therefore, re-infections became more common. Since Christmas, a growing proportion of new SARS-CoV-2 infections have been observed due to the B.1.1.7 strain. These mutations that appeared within the SARS-CoV2 virus genome and the social activities/ contacts between people without obligatory precautions may be the main cause of the third wave [129]. There is a need to increase the vaccination rate to combat this pandemic and adhere to other precautionary public health measures like wearing a mask, using sanitizer, and creating awareness among people regarding social distancing, etc.

Vaccines platform for SARS-CoV-2 infection

The first step regarding managing this viral infection is to ensure adequate isolation to prevent transmission to another individual as transmission rates of SARS-CoV-2 are shown to be higher than other coronaviruses, therefore considered a concern for public health; the majority of countries worldwide adopted different precautionary measures including lockdown policy to reduce the transmission rate via reducing social activities and contacts, that causing a significant negative impact on the economics of different societies all over the world, therefore, there was an urgency to develop safe and

efficacious vaccines to mitigate the current viral disease. In this regard, clinical researchers throughout the world performed intense research and significant progress has been made in the development of efficacious vaccines against SARS-CoV-2 to combat the pandemic.

Currently, several platforms are being utilized globally for vaccine development, and multiple vaccines against SARS-CoV-2 have either been developed or are in the process of being developed in various countries around the world including the USA, Germany, Australia, UK, China, Austria, France, India and Hong Kong (WHO 2020).

Protein subunit

Protein subunit vaccines based on limited fragments of pathogen-drive protein (antigen) to elicit an immune response against pathogen [130], are therefore considered safer than full pathogen-based vaccines. This vaccine is manufactured by DNA recombinant technology but requires an adjuvant to enhance antigenic-specific immunity [131–133]. Many research institutes developed the protein subunit vaccine against SARS-CoV-2 by using spike S-glycoprotein of SARS-CoV-2, and its fragments S1, S2, and RBD as prime targets antigen for subunit vaccine to induce neutralizing antibodies against pathogen [133,134]. Considering the advantages of safety and cost-effectiveness, subunit vaccines are the best option in conjunction with other approaches of vaccine. However, their limitation includes the need for multiple booster administration and adjuvants to potentiate long-term vaccine-induced immune responses [131,132,135].

Novavax NVX-CoV2373

It is a subunit vaccine that was co-developed by the American biotechnology Novavax and the Coalition for Epidemic Preparedness Innovations Foundation. This vaccine contains a recombinant full-length SARS-CoV-2 spike glycoprotein adjuvant with saponin based Matrix-M and manufactured in the established baculovirus-Spodoptera Fruiperda (sf9) insect cell expression system [136–139]. This vaccine is capable of producing a strong antibody response and activating the T cells [140]. Results of phase 3 clinical trials showed that Novavax provided 96.4% protection against symptomatic COVID-19 and 86.3% against the Alpha variant [141]. Clinical trials conducted in the UK indicated that the efficacy of Novavax was 89.7% [142]. On 12 March 2021, Novavax revealed that the COVID-19 vaccine was found to be 96.4% and 86% effective against the original strain and Lineage B.1.1.7, respectively [143].

EpiVacCorona vaccine

This subunit vaccine was manufactured by the VECTOR Institute based on three chemically synthesized peptides of the S glycoprotein, expressed as a chimeric protein [144,145]. Rout of administration of this vaccine is intramuscularly in two doses 3 weeks apart. A person aged 18 years and above is eligible for this vaccine [140]. The phase 1/2 clinical trials showed that EpiVacCorona is safe and immunogenic. The immunological effectiveness of the EpiVac corona vaccine was found to be 100% [144].

Anhui Zhifei (ZF 2001) COVID-19 vaccine

ZF2001 was co-developed by the Chinese Anhui Zhifei Longcom and the Academy of Military Medical Sciences [145]. This subunit vaccine contains the dimeric form of the RBD of the S protein and a conventional alum adjuvant [146]. This vaccine is administered intramuscularly in three doses after every four weeks. Results of phases I and II showed that the vaccine protein subunit was well tolerated and highly immunogenic [145]. Another report showed that RBD-vaccine retained neutralizing effects against Beta and Gamma variants and maintained its neutralizing activity against the Delta strain [147].

mRNA vaccine

mRNA vaccines contain RNA molecules that encode the pathogen's protein or antigen to trigger a protective antigen-specific immune response in the human body. This vaccine has emerged as a noninfectious and nonintegrating platform for vaccine development with no potential risk of infection or insertional mutagenesis. mRNA vaccine is a promising alternative to traditional vaccine approaches due to their safety, potency, strong immunogenicity, low-cost production, fast manufacturing, inherent adjuvant qualities, and unique administration system [148,149]. Unlike DNA vaccines that need to enter the nucleus, this vaccine only needs to enter the cytoplasm to achieve target antigen expression. RNA molecules are delivered via lipid nanoparticles (LNPs) directly into the host cell and mRNA sends instructions to the ribosome in the cytoplasm of the human body cell that is translated into the target protein [150,151]. Once the process is complete, mRNA is digested by ribonuclease [152,153]. As mRNA does not cross through the nuclear membrane, therefore, the risk of unanticipated long-term expression and genetic integration is eliminated [150,154].

Currently two mRNA vaccines, namely, BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna biotechnologies Inc.) were approved by the FDA for emergency use as a prevention against SARS-CoV-2 infection. Both vaccines, BNT162b2 and mRNA-1273 are novel lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine that encodes for a full-length, prefusion stabilized spike (S) protein of SARS-CoV-2. These LNPs form a solid lipid complex that encapsulates and stabilizes

the mRNA and promotes its intracellular uptake [155,156].

BioNTech-Pfizer COVID-19 vaccine (BNT162b2)

BNT162b2 was prepared by BioNTech with support from the pharmaceutical company Pfizer [157]. The mRNA is packaged in a lipid nanoparticle and encodes the entire spike protein [157,158]. The preliminary data from phase 2/3 clinical trials demonstrated that BNT162b2 had 95% efficacy in preventing symptomatic SARS-CoV-2 infections [157]. Moreover, recent studies reported that the BNT162b2 vaccine provides strong protection (≥95%) against the COVID-19 variants detected in the United Kingdom (B.1.1.7) and South Africa (B.1.351) [159,160]. According to another study, the vaccine effectiveness of two doses of the BNT162b2 was found to be 93.7% and 88.0% against the reported infection for the Alpha and Delta variants, respectively [161]. Another study from the United Kingdom found that the Pfizer BioNTech-Comirnaty vaccine had 90% effectiveness against infection for Delta ≥14 days following the second dose [162]. Moreover, this vaccine is 75% effective against documented infection for the Beta variant and retains broad efficacy against the Gamma variant in people 80–96 years of age [159,163].

Moderna vaccine-mRNA-1273

The mRNA-1273 vaccine was developed by Moderna Biotechnologies Inc. and the National Institute of Allergy and Infectious Diseases. The mRNA is encapsulated in lipid nanoparticles (LNPs) and encodes the entire 1273 amino acid sequence of protein spike (S) protein of SARS-CoV-2 virus and is therefore named mRNA-1273 vaccine. The results of a phase III clinical trial showed that two doses of the Moderna-mRNA-1273 vaccine were 94.1% (95% CI: 89.3–96.8) effective against COVID-19 infection [164]. Another study from Spain also demonstrated that the effectiveness of the Moderna-mRNA-1273 vaccine was 86% (95% CI: 56–95) against infection with Alpha variants [165] the vaccine was found to provide continuous protection against infection for the Beta variant [166]. According to the results of another study, the vaccine effectiveness of the second dose was found to be 95.5% (95% CI: 90.9–97.8) against infection ≥14 days for the Gamma variant. Whereas, the results of a negative test case-control study demonstrated that the vaccine was found to be highly effective against infection for the Delta variant 14 to 60 days following the second dose (VE: 94.1%, 95% CI: 90.5–96.3), while it declined to 80.0% (95% CI: 70.2–86.6) for 151–180 days following the second dose [167]. According to the retrospective cohort study from the Czech Republic, the vaccine effectiveness of two doses was found to be 47% (95% CI: 45–49) against reported infection for the Omicron variant [168]. Further studies are needed to confirm vaccine effectiveness against the emerging COVID-19 variants.

CureVac N.V. CureVac/CVnCoV vaccine

Another vaccine named CVnCoV Vaccine is manufactured by CureVac biotech firm in association with Bayer Pharmaceutical Company. This vaccine competes with the Moderna and Pfizer-BioNTech vaccines and has a natural, nonchemically modified synthetic mRNA that encodes the full-length S glycoprotein [142]. Data from phase 1 clinical trials showed that CVnCoV was safe and tolerable in all participants [169]. Results of the phase 2b/3 clinical trials indicated that the vaccine is 48.2% effective against symptomatic coronavirus infection cases, 70.7% effective against moderate-to-severe infection, and 100% against hospitalization and death [170].

DNA vaccine

DNA-based vaccine inserts delivering gene or its fragment (encoding a viral antigen) into the host cells especially the antigen-presenting cells (APC) by using a DNA-plasmids vector. Once, genetic material delivered by this vaccine is translocated to the host's cell nucleus; the mammalian promotor present in the vector is then activated and elicits the transcription and translation of the target translocated gene into protein through host cell machinery [171,172]. The method used to inject plasmid DNA (as a vector) into the cell is electroporation which produces brief electrical pulses to reversibly open small pores in the cell membrane to allow the entrance of plasmid DNA into the cell. This vaccine approach stimulates humoral as well as cell-mediated immune responses efficiently [171,173]. DNA vaccines are noninfectious, easy to produce, cost-effective, and stable. However, DNA vaccine needs to cross the nuclear membrane to become translated which is a major limitation of this vaccine. It may produce antibodies against itself which is another disadvantage of this approach [174]. Recently DNA vaccines have been developed against the SARS-CoV-2 virus by different research groups.

INO-4800 vaccine

Inovio Pharmaceuticals developed a DNA vaccine named INO-4800 that encodes the S protein of the SARS-CoV-2 virus which is used as an antigen of DNA vaccine. Moreover, to improve the DNA vaccine potency, Inovio's DNA medicines deliver plasmids intradermally by electroporation device (CELLECTRA). An electric pulse is then applied to reversibly open small pores in the cell membrane so that the plasmid can enter the cells [175,176]. The preliminary data demonstrated INO-4800 as a potential COVID-19 vaccine candidate, supporting further translational study [177]. On December 14, 2021, Inovio released Clinical Data on Phase 1 and 2 trials that demonstrated INO-4800 appears safe and tolerable as a primary series and as a booster with the induction of both humoral and cellular immune

responses. In addition to eliciting neutralizing antibodies, INO-4800 also induced T-cell immune responses as demonstrated by IFNγ ELISpot [178].

Zydus Cadila ZyCoV-D COVID-19 vaccine (ZyCoV-D)

It is the first plasmid DNA vaccine for human use, prepared by Zydus Cadila, India. It comprises a DNA plasmid vector pVAX1 carrying the gene encoding the S glycoprotein and the sequence encoding for the IgE signal peptide. ZyCoV-D is the first COVID-19 vaccine approved for young adults older than 12 years [179–183]. The DNA vaccine candidate was found to be safe and tolerable during the phase 1 part of the phase 1/2 clinical trials [183]. Further, Interim data from the phase 3 clinical trial reported an efficacy of 66.6% against symptomatic cases [180].

Inactivated whole-virus vaccine

Inactivate virus vaccine contains a complete virus that is inactivated by chemical, radiation, or heat. These vaccines are fully recognized by the immune system resulting in a powerful immune response. Vaccines against hepatitis A, flu, polio, and rabies are examples of inactivated virus-based vaccines [184,185]. As contains inactivated or killed virus, this vaccine cannot replicate and is noninfectious and safer. These vaccines are less potent and provide weaker immunity than live vaccines, so the use of adjuvants and multiple boosters of inactivated vaccines are required to achieve an effusive immune response [186].

Currently, several inactivated virus vaccines are being developed to treat COVID-19. According to the WHO draft landscape of SARS-CoV-2 candidate vaccines, 12 inactivated virus vaccines (14%) are currently in the clinical phase of testing. For instance, pharmaceutical companies like Sinovac and Sinopharm, have produced inactivated viral vaccines that are currently in phase 3 and 4 of clinical trials respectively [187].

Sinovac-CoronaVac COVID-19 vaccine

The Sinovac-CoronaVac vaccine, developed by Sinovac Research and Development Co., Ltd, is an inactivated vaccine created from Vero cells that have been inoculated with SARS-CoV-2 [188]. To determine the efficacy of the vaccine, two double-blinded placebo control studies have been performed in Brazil and Turkey. These trials demonstrated that the vaccine efficacy rate for COVID-19 prevention was 53% in Brazil and 83% in Turkey [189,190]. Further, Phase 3 studies in Turkey and Indonesia showed a protective efficacy of 83.5% and 65.3%, respectively [191,192]. Another phase 3 study in Brazil showed a 50.4% protective efficacy in preventing symptomatic infections, 78% protective efficacy in preventing mild cases, and 100% protective efficacy in the prevention of severe cases [193]. Recent studies revealed that the vaccine is highly effective in neutralizing and nAb activity was significantly reduced against B.1.351 and P1 by a factor of 5.27 and 3.92, respectively [194,195]. Another study revealed the nAb activity was found to be reduced against the P1 lineage [196]. According to one report, CoronaVac has an estimated effectiveness of 59% against Delta [197]. One retrospective cohort study from Chile demonstrated that the efficacy of the Sinovac-CoronaVac vaccine was 37.9% against the Omicron variant among children aged 3–5 years ≥14 days following the second dose [198]. According to another study conducted in Colombia, the vaccine was found to be 67.2% effective at preventing hospitalization and 77.1% effective at preventing death [199].

Covaxin vaccine (Bharat Biotech BBV 152)

BBV 152 is a vero cell-based whole- virion inactivated SARS-CoV-2 vaccine that was developed by Bharat Biotech from an isolated NIV-2020-770 strain of a patient with COVID-19 sequenced in India [200]. BBV152 showed substantial neutralizing antibody responses in the phase 1 study, which persisted up to 3 months following the second vaccination. In contrast to the phase 1 study, the results of phase 2 trials showed good reactogenicity, safety, and enhanced humoral and cell-mediated immune responses [201]. Phase 3 efficacy study reported that the vaccine efficacy was found to be 81%. Covaxin also shows promising results in neutralizing the new UK strain B.1.1.7 [201,202]. Other studies also demonstrate that the vaccine has a neutralizing effect approx. 3 and 2.7 fold for the new variants B.1.351 and B.1617.2 respectively [203,204]. According to phase III analysis of Covaxin on 127 symptomatic cases, the vaccine showed 81% efficacy against mild and moderate COVID-19 disease and against severe COVID-19 disease was 100%. Although the efficacy against asymptomatic COVID-19 infection was found to be 70% [205]. Phase 3 trial conducted in India showed that the vaccine was 77.8% efficacious against symptomatic cases and confers 65.2% protection against the Delta variant [206].

Sinopharm vaccine (BBIBP-CorV COVID-19 vaccine)

This vaccine is developed by the Sinopharm's Beijing Institute of Biological Products (China). China has approved the Sinopharm multivariant vaccine (Sinopharm's BBIBP-CorV) for widespread use, which is a WHO-approved inactivated virus vaccine. In this technology, vero-cell cultivated and inactivated forms of the virus are used. Phase I and phase II randomized clinical trials for the BBIBP-CorV vaccine, have shown that the vaccine is safe and well tolerated at all tested doses [207]. According to the data provided by the WHO regarding phase III, the vaccine is 78.1% effective against COVID-19 (symptomatic) [208]. UAE's MOHAP

previously announced interim results that revealed the vaccine provided 86% efficacy [209]. According to one study conducted in China, the efficacy of the vaccine is 79.34%, and it is 100% effective in suppressing moderate to severe COVID-19 cases [152]. In addition, Sinopharm has started a phase III trial in several countries in Africa, Asia, and Europe [210–212].

Viral vector vaccine

A viral vector vaccine contains a safe modified virus as a viral vector, either replicating or nonreplicating that delivers the genetic code into the body to stimulate both the cell-mediated and humoral immune response. These vaccines have a great potential for prophylactic solution against pathogens as these vaccines trigger the cytotoxic T-cell which leads to the elimination of infected cells [213]. Adenovirus (different from SARS-CoV-2) is being used in these vaccines as a viral vector to introduce transcribed DNA segments of SARS-CoV-2 encoding for the spike glycoprotein (antigen) into the host cells [152,214]. After vaccination, an injected vector delivers genetic code into the body cell to make spike glycoprotein and further activate the immune system [215]. Once spike protein is recognized by the immune system, it starts generating specific neutralizing antibodies followed by T cell activation which in turn destroys spike protein and eliminates the infected cell [150,214]. Various viral vector vaccines are developed to treat coronavirus infection such as Oxford-AstraZeneca vaccine (AZD1222, ChAdOx1 nCoV-19), Sputunik-V vaccine (Gam-COVID-vac), Johnson and Johnson vaccine (Ad26.CoV-2-S) and AD5-nCoV vaccine (Convidacia) [216].

Oxford–AstraZeneca vaccine (AZD1222 COVID-19 vaccine)

This vaccine is a recombinant vaccine, often referred to as ChAdOx1 nCoV-19, manufactured by Oxford University in association with AstraZeneca [145,150,214]. This vaccine is constructed from a nonreplicated chimpanzee adenoviruses vector (ChAdOx1) to transmit SARS-CoV-2 spike proteins to induce an immune response [217]. The AZD1222 vaccine has been approved by the WHO and is used in many countries worldwide [218]. Clinical trials performed on over 60,000 adult participants in the UK, Brazil, South Africa, Kenya, the USA, India, and Japan demonstrate that the vaccine has a safety profile with no serious adverse effects related to the vaccine [219]. According to analysis data from four ongoing blinded, randomized, controlled trials done across the United Kingdom, Brazil, and South Africa, the vaccine showed an overall efficacy of 70.4% (95% CI: 54.8–80.6) for symptomatic COVID-19. According to another analysis in the United Kingdom, Brazil, and South Africa, the efficacy of the AZD1222 vaccine was found to be 81.3% in participants who received two doses spaced within $\geq$12 weeks [220]. A report from the UK study showed that the AstraZeneca vaccine is 74.5% and 67.0% effective against the Alpha and Delta variants, respectively [221]. Further, results of the study from the United Kingdom showed the vaccine efficacy of AZD1222 against documented infection was found to be 73% (95% CI: 66–78) [222]. Another report showed the effectiveness of a double-dose AZD1222 vaccine was 70.4% (95% CI: 43.6–84.5) against symptomatic infection for the Beta variant (B.1.351) [223]. For gamma variants, the effectiveness of a double-dose of this vaccine was found to be 88.1% (95% CI: 82.8–91.7) in a study from Brazil [224] and results of another study showed its effectiveness is 77.9% against gamma variant [225]. Another study from the Czech Republic revealed that the VE of AZD1222 was 51% (95% CI: 23–69) against documented infection for the Omicron variant [226].

Sputnik V vaccine (Gam-COVID-Vac)

Sputnik V was manufactured by the Gamaleya Research Institute of Epidemiology and Microbiology (Russia's Ministry of Health). This is a heterologous adenoviral vector-based vaccine constructed from two vector components, recombinant adenovirus type 26 (rAd26) and recombinant adenovirus type 5 (rAd5) which carry -CoV-2 gene for SARS-CoV-2 full-length glycoprotein S [227]. The gene encoding the S-protein of SARS-CoV-2 carried a rAd26 vector (first dose) that provides humoral and cellular immunity, and the rAd5 vector (second dose) induces the formation of memory cells. From the results of phase 3 trials, the efficacy after 3 weeks of the first dose of the vaccine was found to be 92% [228]. Recently, results of neutralizing activity analysis of Sputnik V vaccine sera against SARS-CoV-2 variants showed that Sputnik V vaccine efficiently neutralized the S protein of the B.1.1.7 variant and the B.1 lineage [229], another report indicated that the vaccine was around 90% effective against the Delta variant [230]. An Interim analysis of the phase 3 study showed that the efficacy of Sputnik V was 91.6% against symptomatic COVID-19 [227]. According to another report, the vaccine showed a protective efficacy of 91.6% against COVID-19 after a complete dose which is comprised of 2 doses given 3 weeks apart intramuscularly [228].

Janssen-Ad26.COV 2.S vaccine

The Janssen-Ad.26.COV2.S vaccine developed by the Janssen Pharmaceutical Companies of Johnson & Johnson is a recombinant adenoviral vector vaccine. It contains a recombinant, replication-incompetent adenovirus serotype 26 (Ad26) vector encoding a full-length and stabilized SARS-CoV-2 spike protein [231,232]. It induced powerful neutralizing antibody responses and provided almost complete protection following SARS-CoV-2 infection [232,233]. This vaccine is 66% effective in preventing disease after a single dose and is capable of suppressing 85% of severe COVID-19 illnesses within

28 days postvaccination [150,152]. An interim analysis of the phase 3 trial showed that vaccine efficacy was 66% against symptomatic disease [234]. A study from Spain also demonstrated the effectiveness of Janssen-Ad26.COV2.S vaccine was estimated to be 77% (95% CI: 27–93) against infection among close contacts with Alpha-infected patients [166]. The vaccine is also highly effective against the B.I.351 lineage observed in South Africa [142]. According to one report, one dose of the Janssen-Ad26.COV2.S vaccine provides effective protection against COVID-19 when the Beta variant is predominant [235]. Results of one study also revealed that the efficacy of Janssen-Ad26.COV2.S vaccine against the symptomatic disease, hospitalization, and death due to the Gamma variant in Brazil was found to be 50.9%, 72.9%, and 90.5% respectively [236]. The efficacy of the vaccine against infection for the Delta variant demonstrated by a study from Spain was estimated at 42% which is lower than that of the Alpha variant [166]. Moreover, the study from the Czech Republic showed that the Janssen-Ad.26.COV2.S vaccine was 60% effective against documented infection for the Omicron variant $\geq$14 days following the second dose [226].

AD5-nCoV vaccine (Convidecia/CanSino)

AD5-nCOV, trade name Convidecia, was developed by CanSino Biologics in cooperation with the Academy of Military Medical Sciences. It is a single-dose viral vector vaccine for COVID-19 [237]. It consists of a recombinant, replication- defective adenovirus type-5 vector (Ad5) expressing the full-length spike protein of SARS-CoV-2 [150]. Data collected from the phase 1 clinical trial suggested that this vaccine is safer and can induce both humoral and cellular responses [238]. CanSino Biologics announced that the interim analysis data of the phase III clinical trial of Convidecia demonstrated that the efficacy vaccine was found to be 65.28% in preventing all symptomatic COVID-19 disease 28 days after single-dose vaccination and an efficacy of 90.07% in preventing severe disease 28 days after single-dose (https://www.precisionvaccinations.com/vaccines/convidicea-vaccine). Moreover, results from the Pakistan subset trial demonstrated that AD5-nCOV is 65.7% effective in preventing symptomatic cases and 100% effective in preventing severe disease [239].

Live attenuated vaccine

These vaccines are developed from attenuated, weakened, or less virulent forms of pathogen to induce strong and long-lasting cellular and humoral immunity. LAV is the most immunogenic vaccine that does not require an adjuvant to achieve optimal response due to its effectiveness in mediating immunity by mimicking natural infection [240]. This vaccine cannot be given to immunocompromised patients, infants, and elderly people due to the risk of reversion of virulent strain [241]. Currently, a live attenuated vaccine named COVI-VAC using deoptimization technology developed by Codagenix Biotech Inc. in collaboration with Serum Institute of India [242]. This is also reported; the University of Hong Kong developed an LAV influenza-based vaccine strain with the deletion in the NS1 gene [243].

Virus-like particle based vaccine

Virus-like particle (VLP) are virus particles that are formed by structural viral protein which are self-assembled into protein nanoparticles and mimic the overall structure of a virus/pathogen. They are noninfectious and nonreplicative as they lack genomic components [244–246]. Because no viral inactivation step or live viruses are involved in the production of the VPL vaccine, VPL can be easily developed in a low-containment production facility [247]. Particle size and the repetitive antigenic surface of the VPL vaccine elicits a robust antibody response by cross-linking the B-cell surface receptor [248–250]. VLP vaccine technology was utilized to develop against SARS-CoV-2. Currently, there are 9 VLP vaccines have been developed against SARS-CoV-2, among which five are being evaluated in clinical trials. The main antigenic component of these vaccines is the S protein, specifically the RBD, which is involved in viral entry and antibody neutralization [251,252]. Recently, the Central Committee on Research Involving Human Subjects in the Netherlands approved an ABNCoV2 capsid virus-like particle-based COVID-19 vaccine developed by AdaptVac, a PREVENT-nCoV consortium member [253].

COVID-19 pandemic in Japan

The first case of COVID-19 in Japan was recognized by the government on January 16, 2020 and the first death was recorded on 14 Feb of the same year. The vaccination was started on February 17, 2021, Japan approved Pfizer–BioNTech, Moderna, and Oxford–AstraZeneca for use. The number of confirmed cases of COVID-19 in Japan reached 33 803 574 with 74 694 deaths and 33 728 878 recovered [254]. According to the medical task-force advice to the government, three important issues to deal with are; early detection and response to clusters, early patient diagnosis and intensive care for the severely ill, and behavior modification of citizens [255]. Cluster Response Team (Ministry of Health, Labor and Welfare) was established in February 2020 to identify small-scale clusters of COVID-19 infections before they grow into mega-clusters. The Japanese government also focuses on the behavior modification of citizens to train them to comply with the voluntary state of emergency measures

[256]. The extreme caution when contacting the elderly, work style changes such as remote work, avoidance of close contact settings, wearing masks, refraining from talking on public transportation, and long-distance traveling were also taken into consideration. In May 2023, Japan decided to downgrade the status of the coronavirus from strict category 2 (which includes tuberculosis) to category 5 of common infectious diseases and seasonal flu [257]. The number of new patients has been on a gradual upward trend since early April 2023 and has continued to increase, while in Okinawa Prefecture the numbers have been declining since early July. The number of new patients across the country by age group is increasing in all age groups except for those in their teens. As for the mutant strains, XBB.1.9 strains are unchanged, XBB.1.16 strains and XBB.2.3 strains increasing, and XBB.1.5 strains decreasing [258].

Designed a plasmid-based nasal COVID-19 vaccine at HIC clinic, Japan

Nasal vaccines offer new benefits for use and efficacy, as they are easy to deliver and can be used by the individual without medical staff assistance by using a suitable device for the nasal application, with minimum discomfort [259]. Another advantage is that nasal vaccination induces both mucosal and systemic immunity while intramuscular vaccination primarily induces systemic immune response [260].

We in our molecular biology laboratory at HIC-Clinic construct a plasmid-based COVID-19 vaccine that can be administrated via the nasal route. SARS-CoV-2 can be transmitted by respiratory particles released from an infected subject, aerosol from the infected subjects, due to their smaller diameter (<5 μm) can infect subjects at higher distances via the nasal entry [261]. The mucous membrane of the nose and throat is an important component of the immune system, one of the first lines of defense against germs entering from outside the body. It contains antibacterial enzymes found in the mucus, cilia move out the mucus from the airway and they also contain antibodies most important of which is IgA. The SARS-CoV-2 virus first infects the nose and throat before spreading to the lungs, where severe COVID-19 can develop. This entry gate of the nose and throat is always exposed to the fresh attacker virus from the environment, even after the development of the blood antibodies after the usual injected vaccines, these entry points are difficult to reach, and the injected vaccines often fail to prevent the virus infection through the upper respiratory tract [262]. Nasal vaccine application can induce both mucosal and systemic immune responses [263]. Secretory IgA (dimeric form) is the main immunoglobulin found in mucous secretions and also in small amounts in blood [264]. In response to luminal stimulation by vaccines, antigen-presenting cells in epithelial cells, Peyer's patches, and lymph follicles will produce cytokines and stimulate the B-cells to produce IgA [265]. Helper T Cells and NKT cell-derived cytokines play an important role in the production of high-affinity IgA [259]. Spike (S) glycoprotein has a crucial role in virus attachment and entry by binding angiotensin-converting enzyme 2 (ACE2) receptors through its receptor-binding domain, and this S protein is being widely used as a target for vaccine development. Based on our experience in the development of anticancer gene therapy and plasmid-based cancer vaccines [266], we designed recombinant COVID-19 vaccines and tested them for their efficacy. The plasmid construction for the expression of SARS-CoV2 spike protein was performed under an enhanced Human cytomegalovirus immediate-early (CMV) promoter for high-level expression of recombinant proteins in mammalian cells. Here the ampicillin resistance gene plasmid was used, and the gene insert sequence for surface glycoprotein was selected as is available from the NCBI database (GenBank: MN908947.3). Following Fig. 1 demonstrates the cellular and humoral response elicited after the coronal nasal DNA vaccine.

Delivery device of HIC clinic nasal COVID-19 vaccine

We developed a delivery device for nasal vaccines with already-in-use clinical-grade components and rapping. The components are shown in Fig. 2, and the main filling body is one ml syringe of the NIPRO Japan.

Challenges and future perspectives

The COVID-19 pandemic has spread globally and become the world's most lethal threat and contagious disease to the health of the global population, especially with the emergence of variants of the SARS-CoV-2 virus. These immune evasive variants integrate the mutation to the spike protein which could affect immune recognition of antibodies derived from existing vaccines and hinder the therapeutic antibody function thus affecting the degree of protection by vaccines [267]. Hence, questions have been raised concerning the effectiveness of developed vaccines against new variants. Moreover, previous studies on coronaviruses also showed that various mutations in the target proteins of the coronaviruses can be associated with drug resistance and changes in the structures of target proteins that may lead to vaccine inefficacy [23]. Therefore, the uncertainty over long-lasting protection against COVID-19 has also risen. It is crucial to determine how long a protective immune

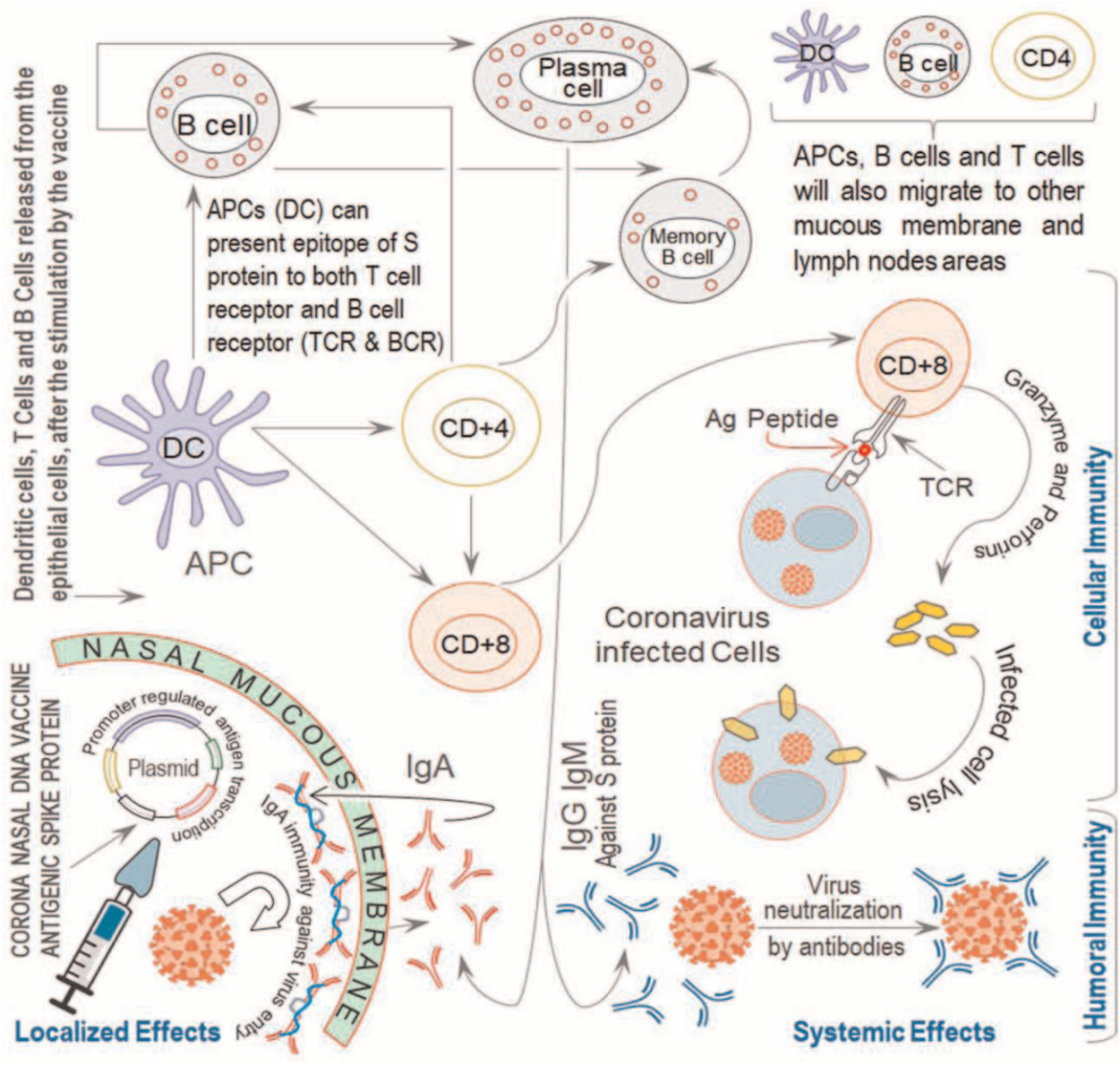

Fig. 1. Demonstration of local effect and systemic after coronal nasal DNA vaccine.

response can be maintained in an individual after vaccination [268,269]. Because some cases of patients with reinfection have been reported who had recovered from COVID-19. Ideally, vaccination should induce long-term immunity, but annual vaccination would be feasible based on experiences with the annual influenza vaccine [270,271]. To induce long-lasting protective immunity, the additional boosting dose of an updated vaccine is required especially for those who are immunocompromised, and have concomitant comorbidities to enhance antigen-specific immunity [131]. To improve the vaccine's protective efficacy adjuvant with better immunogenicity may play an important role [272]. In addition, vaccine effectiveness affected by immune-evasive variants could improve with the development of multiple epitope-based vaccines, so in case one epitope does not respond the other could take over and conclusively vaccine efficacy could enhance [253]. Nevertheless, considering the continuous emergence of new variants, pursuing research on variants, developing long-term studies, and clinical trials on a large scale are pivotal in determining the efficacy of existing vaccines against variants and the currently authorized vaccines may need to be updated periodically to avoid clinical efficacy loss (Figs. 3–5).

Another great challenge due to the mutation in spike protein is the increase in the transmissibility of virus variants. Human-to-human transmission of SARS-CoV-2 is highly observed even in hospital settings, although strict isolation and quarantine measures are ensured at medical facilities [51,273]. Recently, 41% of the patients

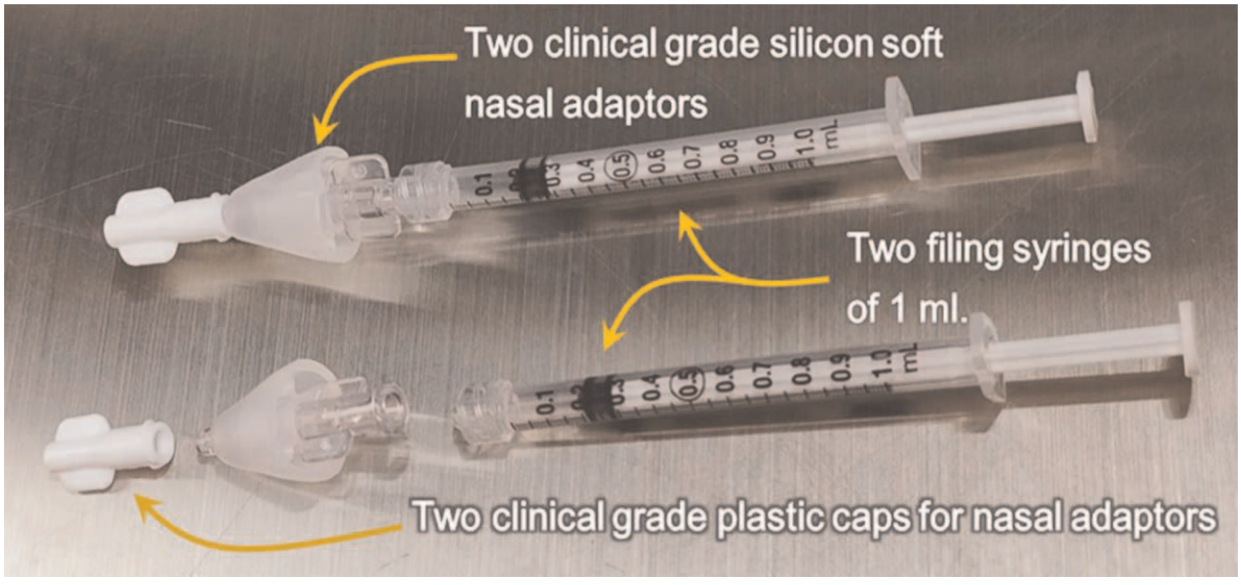

Fig. 2. Nasal COVID-19 vaccine delivery device components. Photograph of an un-filled nasal vaccine device to show its components in a clean bench. The upper device is a complete and final form with all three components attached. Below is an exploded image of the device with all three components separated.

were found to be infected in hospital settings, out of which 29% were medical staff [274]. Transmission in hospital and healthcare settings poses a very serious threat and requires strict vigilance to control virus transmission. It is crucial to track the SARS-CoV-2 mutations/variants, and countries all around the world should increase the attention to disease surveillance systems and scale up response operations to control the spread of the SARS-CoV-2 virus and keep strict attention on following all the preventive measures must be continued by every individual to control the spread of SARS-CoV-2 virus. Most importantly, advocates vaccinating unvaccinated individuals as a priority in suppressing the transmission and mutation of SARS-CoV-2 and guiding them to follow hygiene guidelines after vaccination is a vital strategy that may contribute to better limiting SARS-CoV-2 spread [275].

Another challenge that may become a matter of concern for the prevention of diseases by vaccination is the antibody-dependent enhancement (ADE) phenomenon. ADE exists in several kinds of viruses. Viral infection

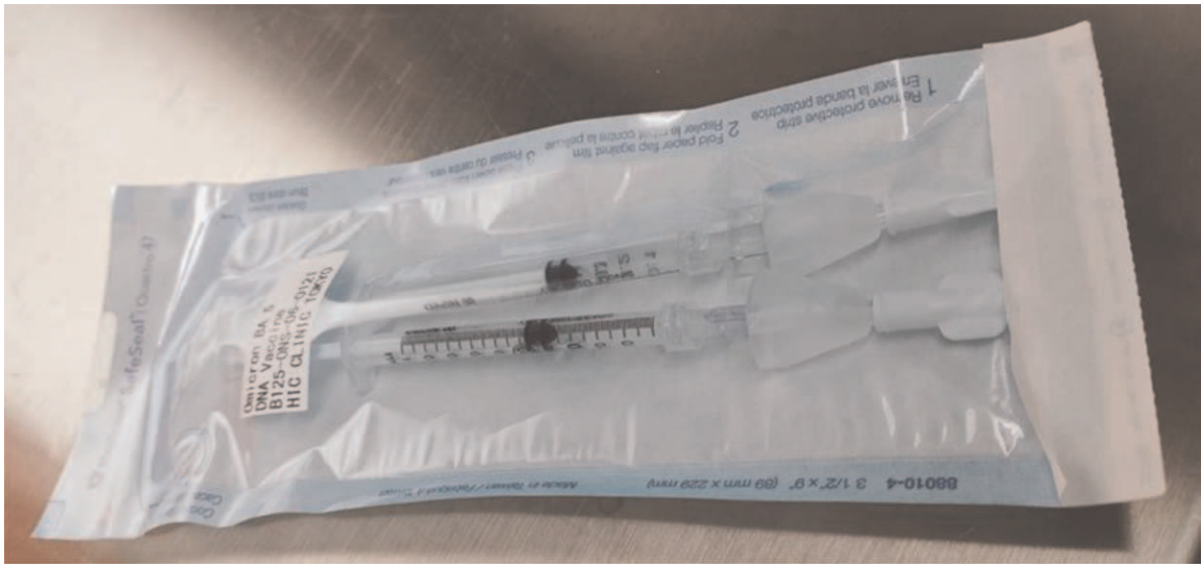

Fig. 3. Nasal COVID-19 vaccine delivery device in packaging envelope. Photograph of a filled device in its sterilized envelope with a label for the description and date of the drug inside.

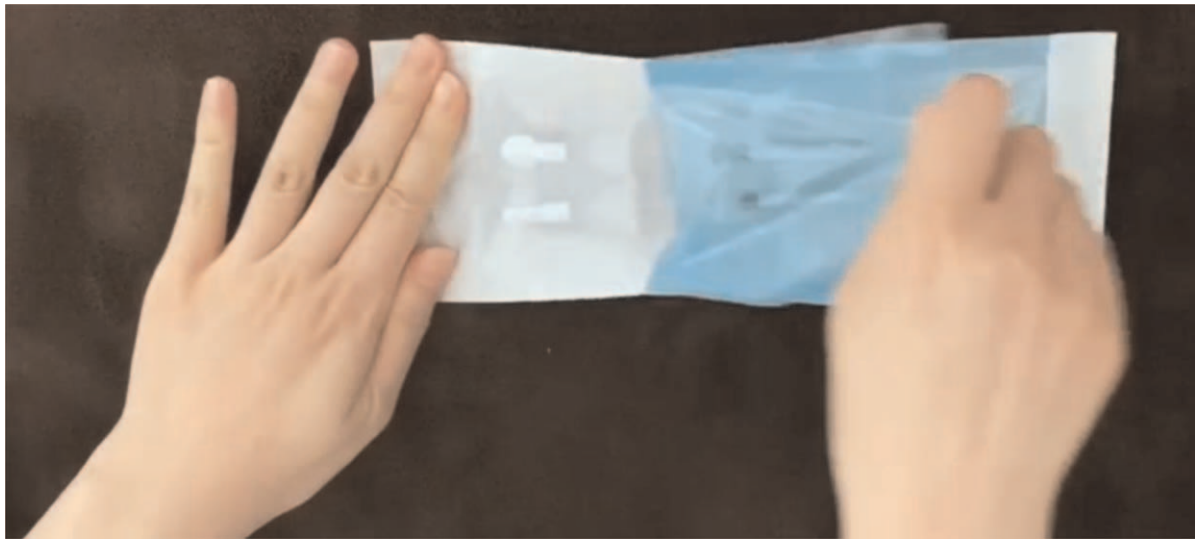

Fig. 4. Nasal COVID-19 vaccine delivery device unpacking from the packaging. Photograph of a filled device before use. The surface or table used should be clean and sterilized, as well as the hands of the person involved.

initiates with the attachment of the virus particle to the cytoplasm membrane on the cell surface, to block this viral attachment, antibodies are secreted that target the viral surface proteins specifically, and control infections by neutralizing the viruses, weakening their infective ability. However, in ADE incidence, the binding of specific antibodies to viral surface proteins can promote viral invasion into the cell through Fc receptors, thus enhancing viral infection [276]. Previous studies demonstrate that neutralizing antibodies against RBD of MERS-CoV and SARS-CoV facilitated virus entry into Fc receptor-expressing human cells in vitro [277]. In the current pandemic, several specialists have suggested that immune responses against SARS-CoV-2 could lead to ADE [279]. However, the incidence of SARS-CoV-2 ADE has not yet been proven [278]. An alternative target to overcome ADE may be the nonsurface proteins, such as the nucleocapsid (N) protein. Because the N protein is not on the virus surface, its antibodies cannot promote virus entry. Nucleic acid based vaccines such as DNA and RNA-based platforms could quickly provide solutions to bypass such issues, as motifs of the vaccine antigen that cause ADE could be easily engineered or removed [279]. Moreover, another issue is the vaccine development process (up to licensing of the vaccine) which in itself is a long and expensive process and generally, it takes near about 10 years to develop a novel vaccine and requires a huge amount of money [280]. It has been estimated that almost US$31–68 million is required to bring a vaccine candidate to the end of phase-II trials [281]. Most importantly, collaboration is the most significant strategy for therapeutic development and new research [282]. Therefore, collaborations between different representatives from academia and biopharmaceutical companies might help to develop effective vaccines very quickly.

Fig. 5. Nasal COVID-19 vaccine delivery device self-application by the person. Photograph of a filled device during the self-application of nasal vaccine by the person.

Another important concern about vaccines is that vaccine candidates are tested on certain numbers of a population instead of all population groups, therefore different population groups such as elderly, young, special patients, and immunocompromised individuals, pregnant and breastfeeding women are not considered in the initial vaccine research, hence, unwarranted adverse effects may observe in these groups during general and universal use of the vaccine [283]. Another greater challenge regarding vaccination is vaccine hesitancy which is extensively prevalent not only in developing countries but also in technologically advanced countries with a high literacy

rate. Vaccine refusal has been an issue physicians have had to deal with even in prepandemic times. Regarding the COVID-19 pandemic, there has been growing COVID-19 vaccine hesitancy [284,285]. Understanding the vaccine hesitancy is essential to achieve the desired level of immunization. When a vaccine with a high percentage of efficacy, is introduced/used in public, but it does not show the desired effect as expected, peoples erodes confidence in that vaccine. As a result, fewer people accept the vaccine, which makes the pandemic worse. Another reason for developing vaccine hesitancy in public is the spreading of scientific-sound misinformation regarding vaccines. As COVID-19 vaccines have only been recently introduced worldwide, in a much faster period compared to a typical vaccine development [286], acceptance of vaccines against SARS-CoV-2 presented a public health challenge due to false information through social media platforms that vaccine trial participants have died after taking a candidate COVID-19 vaccine. Reduced available data on safety and efficiency also contributed to this issue [287]. Therefore, it is very important to improve vaccine acceptance and reduce vaccine hesitancy, the scientific community and health-care staff build trust and establish effective communication between the people and the public health system and provide them with transparent information regarding vaccine safety and effectiveness [288].

One of the important challenges of vaccination is the issue of inequality in the distribution of vaccines which has affected many people's lives around the world, especially in poor and less developed countries [289]. Developed countries continue to sign agreements with the world's largest pharmaceutical companies to purchase vaccines more than their requirements at prices that are not possible for most countries. Therefore, developing countries face acute problems in obtaining vaccines [290,291]. To overcome the existence of these inequalities, WHO with other international organizations such as the Global Alliance for Vaccines and Immunizations (GAVI) and Coalition for Epidemic Preparedness Innovations (CEPI) have made efforts to establish a system to ensure the right and equitable distribution of vaccine named the COVID-19 Vaccines Global Access (COVAX). In September 2020, the WHO under COVAX outlined the vaccine distribution policy [292]. Though these efforts significantly contributed to building a fair vaccination distribution plan [293], but it has been witnessed globally during the pandemic that income has been an important factor i.e., high-income countries have achieved the fastest vaccine [294].

Another important issue is related to vaccine storage, and transportation, which puts an extra financial burden on developing countries. Most of the vaccines store significantly at low storage temperatures that are below the freezing point, which is difficult to maintain for longer durations and poses a huge limitation in developing countries. Further, vaccines must be protected from direct exposure to sunlight and ultraviolet light which makes their handling more difficult [227]. Despite the rapid development and global distribution of vaccines in various countries, the number of individuals vaccinated still represented a small proportion of the world [295]. Investments in vaccine developments against new variants must be accompanied by investments in planned immunization infrastructure, disease surveillance, and public health to mitigate the devastating effects of COVID-19 pandemic. Public health measures, such as introducing mandatory masks to reduce viral transmission, and creating awareness among people regarding social distancing play a basic role in controlling the spread of infection.

Conclusion

COVID-19 has been a dreadful threat in this era for mankind. To date, several vaccines with high efficacy have been developed in various countries all over the world. Despite the availability of the highly effective vaccine, this pandemic is still causing a high mortality rate, and the recent hit by devastating multiple waves of coronavirus places a significant concern on the state, government, healthcare services, scientists, and researchers in establishing successful and effective vaccines, therapeutics and strategies to cope with coming waves. Evidently, more research is required to improve the efficacy and safety of developed vaccines by increasing neutralizing antibodies because the emergence of multiple variants of the SARS-CoV-2 virus causes an increase in their infectivity, ability to escape host immune response, and decreased neutralization from vaccination. Nevertheless, adherence to precautionary hygiene measures (including hand washing, wearing masks, and social distancing), continuous monitoring, and high throughput sequencing of the SARS-CoV-2 virus genome, and vaccination are crucial steps in the prevention of virus transmission and early detection of variants to combat this pandemic.

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Conflicts of interest

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References

1. Ksiazek TG, Erdman D, Goldsmith CS, Zaki SR, Peret T, Emery S, *et al*. **A novel coronavirus associated with severe acute respiratory syndrome.** *N Engl J Med* 2003; **348**:1953–1966.
2. Khodadadi E, Maroufi P, Khodadadi E, Esposito I, Ganbarov K, Espsoito S, *et al*. Study of combining virtual screening and antiviral treatments of the Sars-CoV-2. (COVID-19). Microb Pathog 2020;145.
3. Najafi K, Maroufi P, Khodadadi E, Zeinalzadeh E, Ganbarov K, Asgharzadeh M, Kafil HS. **SARS-CoV-2 receptor ACE2 and molecular pathway to enter target cells during infection.** *Rev Res Med Microbiol* 2022; **33**:e105–e113.
4. Alsuliman T, Alasadi L, Alkharat B, Srour M, Alrstom A. **A review of potential treatments to date in COVID-19 patients according to the stage of the disease.** *Curr Res Transl Med* 2020; **68**:93–104.
5. WHO. 11 March 2020. Director-General's Opening Remarks at the Media Briefing on COVID-19.
6. WHO. The different types of COVID-19 vaccines. 2021. Available at: https://www.who.int/news-room/feature-stories/detail/the-race-for-a-covid-19-vaccine-explained [Accessed 13 May 2022].
7. WHO. WHO-COVID19 Vaccine Tracker. 2022. Available at: https://covid19.trackvaccines.org/agency/who/ [Accessed 13 May 2022].
8. Ritchie H, Mathieu E, Rodés-Guirao L, Coronavirus (COVID-19) vaccinations – our world in data. Available at: https://ourworldindata.org/covid-vaccinations?country=OWID_WRL [Accessed 13 May,2022].
9. A review of coronavirus disease-2019 (COVID-19). Ind J Pediatr 2020;87:281–6.
10. Richman DD, Whitley RJ, Hayden FG. **Clinical virology.** 4th ed. Washington: ASM Press; 2016.
11. Belmehdi O, Hakkour M, El Omari N, Balahbib A, Guaouguaou FE, Taoufiq B, *et al*. **Molecular structure, pathophysiology, and diagnosis of COVID-19.** *Biointerface Res Appl Chem* 2021; **11**:10215–10237.
12. Abd El-Aziz TM, Stockand JD. **Recent progress and challenges in drug development against COVID-19 coronavirus (SARS-CoV-2) – an update on the status.** *Infect Genet Evol* 2020; **83**:104327.
13. Randhawa GS, Soltysiak MPM, El Roz H, de Souza CPE, Hill KA, Kari L. **Machine learning using intrinsic genomic signatures for rapid classification of novel pathogens: COVID-19 case study.** *PLoS One* 2020; **15**:e0232391.
14. Velavan TP, Meyer CG. **The COVID-19 epidemic.** *Trop Med Int Health* 2020; **25**:278–280.
15. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, *et al*. **A novel coronavirus from patients with pneumonia in China, 2019.** *New England J Med* 2019; **382**:727–733.
16. Asghari A, Naseri M, Safari H, Saboory E, Parsamanesh N. **The novel insight of SARS-CoV-2 molecular biology and pathogenesis and therapeutic options.** *DNA Cell Biol* 2020; **39**:1741–1753.
17. Lei J, Kusov Y, Hilgenfeld R. **Nsp3 of coronaviruses: structures and functions of a large multidomain protein.** *Antiviral Res* 2018; **149**:58–74.
18. Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR. **Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): the epidemic and the challenges.** *Int J Antimicrob Agents* 2020; **55**: 105924.
19. Hazafa A, Ur-Rahman K, Haq IU, Jahan N, Mumtaz M, Farman M, *et al*. **The broad-spectrum antiviral recommendations for drug discovery against COVID-19.** *Drug Metab Rev* 2020; **52**:408–424.
20. Naqvi AAT, Fatima K, Mohammad T, Fatima U, Singh IK, Singh A, *et al*. **Insights into SARS-CoV-2 genome, structure, evolution, pathogenesis and therapies: structural genomics approach.** *Biochim Biophys Acta Mol Basis Dis* 2020; **1866**:165878.
21. Chan JF, Kok KH, Zhu Z, Chu H, To KK, Yuan S, Yuen KY. **Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan.** *Emerg Microbes Infect* 2020; **9**:221–236.
22. Boursnell MEG, Brown TDK, Foulds IJ, Green PF, Tomley FM, Binns MM. **Completion of the sequence of the genome of the coronavirus avian infectious bronchitis virus.** *J Gen Virol* 1987; **68**:57–77.
23. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, *et al*. **Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation.** *Science* 2020; **367**:1260–1263.
24. Abduljali J, Abduljali B. **Epidemiology, genome and clinical features of the pandemic SARS-CoV-2: a recent view.** *New Micr New Infect* 2020; **35**:100672.
25. Parsamanesh N, Pezeshgi A, Hemmati M, Jameshorani M, Saboory E. **Neurological manifestations of coronavirus infections: role of angiotensin-converting enzyme 2 in COVID-19.** *Int J Neurosci* 2020; **132**:917–924.
26. Li H, Liu L, Zhang D, Xu J, Dai H, Tang N, *et al*. **SARS-CoV-2 and viral sepsis: observations and hypotheses.** *Lancet* 2020; **395**:1517–1520.
27. Letko M, Marzi A, Munster V. **Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses.** *Nat Microbiol* 2020; **5**:562–569.
28. Khailany RA, Safdar M, Ozaslan M. **Genomic characterization of a novel SARS-CoV-2.** *Gene Rep* 2020; **19**:100682.
29. Jiang S, Hillyer C, Du L. **Neutralizing antibodies against SARS-CoV-2 and other human coronaviruses.** *Trends Immunol* 2020; **41**:355–359.
30. Song W, Gui M, Wang X, Xiang Y. **Cryo-EM structure of the SARS coronavirus spike glycoprotein in complex with its host cell receptor ACE2.** *PLoS Pathog* 2018; **14**:e1007236.
31. de Abajo FJ, Rodríguez-Martín S, Lerma V, Mejía-Abril G, Aguilar M, García-Luque A, *et al*. **Use of renin-angiotensin-aldosterone system inhibitors and risk of COVID-19 requiring admission to hospital: a case-population study.** *Lancet* 2020; **395**:1705–1714.
32. Chen Y, Guo Y, Pan Y, Zhao ZJ. **Structure analysis of the receptor binding of 2019-nCoV.** *Biochem Biophys Res Commun* 2020; **525**:135–140.
33. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. **Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein.** *Cell* 2020; **183**:281–292.e.
34. Ujike M, Taguchi F. **Incorporation of spike and membrane glycoproteins into coronavirus virions.** *Viruses* 2015; **7**:1700–1725.
35. Fehr AR, Perlman S. **Coronaviruses: an overview of their replication and pathogenesis.** *Methods Mol Biol* 2015; **1282**:1–23.
36. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, *et al*. **Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China.** *Lancet* 2020; **395**:497–506.
37. Rettner R. **How does the new coronavirus compare with the flu?** *Live Sci* 2020; **25**.
38. Hong H, Wang Y, Chung HT, Chen CJ. **Clinical characteristics of novel coronavirus disease 2019 (COVID-19) in newborns, infants and children.** *Pediatr Neonatol* 2020; **61**:131–132.
39. Rothan HA, Byrareddy SN. **The epidemiology and pathogenesis of coronavirus disease (COVID-19) outbreak.** *J Autoimmun* 2020; **109**:102433.
40. Yang Y, Islam MS, Wang J, Li Y, Chen X. **Traditional Chinese medicine in the treatment of patients infected with 2019-new coronavirus (SARS-CoV-2): a review and perspective.** *Int J Biol Sci* 2020; **16**:1708–1717.
41. Esbin MN, Whitney ON, Chong S, Maurer A, Darzacq X, Tjian R. **Overcoming the bottleneck to widespread testing: a rapid review of nucleic acid testing approaches for COVID-19 detection.** *RNA* 2020; **26**:771–783.
42. Wang J, Cai K, Zhang R, He X, Shen X, Liu J, *et al*. **Novel one-step single-tube nested quantitative real-time PCR assay for highly sensitive detection of SARS-CoV-2.** *Anal Chem* 2020; **92**:9399–9404.
43. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DKW, *et al*. **Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR.** *Euro Surveill* 2020; **25**:2000045.
44. Porte L, Legarraga P, Vollrath V, Aguilera X, Munita JM, Araos R, *et al*. **Evaluation of a novel antigen-based rapid detection test for the diagnosis of SARS-CoV-2 in respiratory samples.** *Int J Infect Dis* 2020; **99**:328–333.
45. Tang YW, Schmitz JE, Persing DH, Stratton CW. **Laboratory diagnosis of COVID-19: current issues and challenges.** *J Clin Microbiol* 2020; **58**:e00512–e520.

46. Leeflang MM, Rutjes AW, Reitsma JB, Hooft L, Bossuyt PM. **Variation of a test's sensitivity and specificity with disease prevalence.** *CMAJ* 2013; **185**:E537–E544.
47. Kan B, Wang M, Jing H, Xu H, Jiang X, Yan M, *et al*. **Molecular evolution analysis and geographic investigation of severe acute respiratory syndrome coronavirus-like virus in palm civets at an animal market and on farms.** *J Virol* 2005; **79**:11892–11900.
48. Tu C, Crameri G, Kong X, Chen J, Shun Y, Yu M, *et al*. **Antibodies to SARS coronavirus in civets.** *Emerg Infect Dis* 2004; **10**:2244–2248.
49. Lau SK, Woo PC, Li KS, Huang Y, Tsoi HW, Wong BH, *et al*. **Severe acute respiratory syndrome coronavirus-like virus in Chinese horseshoe bats.** *Proc Natl Acad Sci USA* 2005; **102**:14040–14045.
50. Li W, Shi Z, Yu M, Ren W, Smith C, Epstein JH, *et al*. **Bats are natural reservoirs of SARS-like coronaviruses.** *Science* 2005; **310**:676–679.
51. Chan JFW, Yuan S, Kok KH, To KK, Chu H, Yang J, *et al*. **A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster.** *Lancet* 2020; **395**:514–523.
52. Malta M, Rimoin AW, Strathdee SA. **The coronavirus 2019-nCoV epidemic: is hindsight 20/20?** *EClinicalMedicine* 2020; **20**:100289.
53. Li Q, Guan X, Wu P, Wng X, Zhou L, Tong Y, *et al*. **Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia.** *N Engl J Med* 2020; **382**:1199–1207.
54. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, *et al*. **Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding.** *Lancet* 2020; **395**:565–574.
55. Han Y, Du J, Su H, Zhang J, Zhu G, Zhang S, *et al*. **Identification of diverse bat alphacoronaviruses and betacoronaviruses in China provides new insights into the evolution and origin of coronavirus-related diseases.** *Front Microbiol* 2019; **10**:1900.
56. Cui J, Li F, Shi ZL. **Origin and evolution of pathogenic coronaviruses.** *Nat Rev Microbiol* 2019; **17**:181–192.
57. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, *et al*. **A pneumonia outbreak associated with a new coronavirus of probable bat origin.** *Nature* 2020; **579**:270–273.
58. Xiao K, Zhai J, Feng Y, Xhou N, Zhang X, Zou JJ, *et al*. **Isolation of SARS-CoV-2-related coronavirus from Malayan pangolins.** *Nature* 2020; **583**:286–289.
59. Riou J, Althaus CL. **Pattern of early human-to-human transmission of Wuhan 2019 novel coronavirus (2019-nCoV), December 2019 to January 2020.** *Euro Surveill* 2020; **25**:2000058.
60. Lotfi M, Hamblin MR, Rezaei N. **COVID-19: transmission, prevention, and potential therapeutic opportunities.** *Clin Chim Acta* 2020; **508**:254–266.
61. Carlos WG, Dela Cruz CS, Cao B, Pasnick S, Jamil S. **Novel Wuhan (2019-nCoV) coronavirus.** *Am J Respir Crit Care Med* 2020; **201**:7–8.
62. Ather A, Patel B, Ruparel NB, Diogenes A, Hargreaves KM. **Coronavirus disease 19 (COVID-19): implications for clinical dental care.** *J Endod* 2020; **46**:584–595.
63. Cai J, Sun W, Huang J, Gamber M, Wu J, He G. **Indirect virus transmission in cluster of COVID-19 cases, Wenzhou, China, 2020.** *Emerg Infect Dis* 2020; **26**:1343–1345.
64. Ma Y, Wu L, Shaw N, Gao Y, Wang J, Sun Y, *et al*. **Structural basis and functional analysis of the SARS coronavirus nsp14-nsp10 complex.** *Proc Natl Acad Sci USA* 2015; **112**:9436–9441.
65. Korber B, Fischer WM, Gnanakaran S, Yoon H, Theiler J, Abfalterer W, *et al*. **Tracking changes in SARS-CoV-2 spike: evidence that D614G increases infectivity of the COVID-19 Virus.** *Cell* 2020; **182**:812–827e19.
66. Oreshkova N, Molenaar RJ, Vreman S, Harders F, Oude Munnink BB, Hakze-van der Honing RW, *et al*. **SARS-CoV-2 infection in farmed minks, the Netherlands, April and May 2020.** *Euro Surveill* 2020; **25**:2001005.
67. Rambaut A, Holmes EC, O'Toole Á, Hill V, McCrone JT, Ruis C, *et al*. **A dynamic nomenclature proposal for SARS-CoV-2 lineages to assist genomic epidemiology.** *Nat Microbiol* 2020; **5**:1403–1407.
68. Volz E, Hill V, McCrone JT, Price A, Jorgensen D, O'Toole A, *et al*. **Evaluating the effects of SARS-CoV-2 spike mutation D614G on transmissibility and pathogenicity.** *Cell* 2021; **184**:64–75e11.
69. WHO World Health Organization Weekly Epidemiological Update on COVID-19. 29 December 2021. Available at: https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/ [Accessed 4 February 2022].
70. Galloway SE, Paul P, MacCannell DR, Johansson MA, Brooks JT, MacNeil A, *et al*. **Emergence of SARS-CoV-2 B.1.1.7 lineage – United States, December 29, 2020–January 12, 2021.** *MMWR Morb Mortal Wkly Rep* 2021; **70**:95–99.
71. Volz E, Mishra S, Chand M, Barrett JC, Johnson R, Geidelberg L, *et al*. **Assessing transmissibility of SARS-CoV-2 lineage B.1.1.7 in England.** *Nature* 2021; **593**:266–269.
72. ViralZone SARS-CoV-2 circulating variants. Available at: https://viralzone.expasy.org/9556 [Accessed 7 January 2022].
73. Coronavirus mutations and variants: what does it mean? | SRHD. n.d. https://srhd.org/news/2021/coronavirus-mutations-and-variants-what-does-it-mean [Accessed 13 May 2021].
74. Starr TN, Greaney AJ, Hilton SK, Ellis D, Crawford KHD, Dingens AS, *et al*. **Deep mutational scanning of SARS-CoV-2 receptor binding domain reveals constraints on folding and ACE2 binding.** *Cell* 2020; **182**:1295–1310.e10.
75. How the flu virus can change: ''Drift'' and ''Shift''. Centers for Disease Control and Prevention. Available at: https://www.cdc.gov/flu/about/viruses/change.htm [Accessed April 23, 2021].
76. Fontanet A, Autran B, Lina B, Kieny MP, Karim SSA, Sridhar D. **SARS-CoV-2 variants and ending the COVID-19 pandemic.** *Lancet* 2021; **397**:952–954.
77. Bernal JL, Andrews N, Gower C, Gallagher E, Simmons R, Thelwall S, *et al*. **Effectiveness of Covid-19 Vaccines against the B.1.617.2 (Delta) Variant.** *N Engl J Med* 2021; **385**:585–594.
78. Abu-Raddad LJ, Chemaitelly H, Yassine HM, Benslimane FM, Al Khatib HA, Tang P, *et al*. **Pfizer-BioNTech mRNA BNT162b2 COVID-19 vaccine protection against variants of concern after one versus two doses.** *J Travel Med* 2021; **28**:taab083.
79. Tegally H, Wilkinson E, Giovanetti M, Iranzadeh A, Fonseca V, Giandhari J, *et al*. **Detection of a SARS-CoV-2 variant of concern in South Africa.** *Nature* 2021; **592**:438–443.
80. Wibmer CK, Ayres F, Hermanus T, Madzivhandila M, Kgagudi P, Oosthuysen B, *et al*. **SARS-CoV-2 501y.V2 escapes neutralization by South African COVID-19 donor plasma.** *Nat Med* 2021; **27**:622–625.
81. Planas D, Bruel T, Grzelak L, Guivel-Benhassine F, Staropoli I, Porrot F, *et al*. **Sensitivity of infectious SARS-CoV-2 B.1.7 and B.1.351 variants to neutralizing antibodies.** *Nat Med* 2021; **27**:917–924.
82. Duong D. **Alpha, Beta, Delta, Gamma: what's important to know about SARS-CoV-2 variants of concern?** *CMAJ* 2021; **193**:E1059–E1060.
83. Madhi SA, Baillie V, Cutland CL, Voysey M, Koen AL, Fairlie L, *et al*. **Efficacy of the ChAdOx1 nCoV-19 COVID-19 vaccine against the B.1.351 variant.** *N Engl J Med* 2021; **384**:1885–1898.
84. Sabino EC, Buss LF, Carvalho MPS, Prete CA Jr, Crispim MAE, Fraiji NA, *et al*. **Resurgence of COVID-19 in Manaus, Brazil, despite high seroprevalence.** *Lancet* 2021; **397**:452–455.
85. Dholariya S, Parchwani DN, Singh R, Sonagra A, Motiani A, Patel D. **Notable and emerging variants of SARS-CoV-2 virus: a quick glance.** *Indian J Clin Biochem* 2021; **36**:451–458.
86. Maggi F, Novazzi F, Genoni A, Baj A, Spezia PG, Focosi D, *et al*. **Imported SARS-CoV-2 variant P.1 in traveler returning from Brazil to Italy.** *Emerg Infect Dis* 2021; **27**:1249–1251.
87. COVID-19 epidemic in the Brazilian state of Amazonas was driven by long-term persistence of endemic SARS-CoV-2 lineages and the recent emergence of the new Variant of Concern P.1. Home. Published February 25, 2021. Available at: https://www.researchsquare.com/article/rs-275494/v1 [Accessed April 24, 2021].
88. Glatter R. P.1 variant, dominant strain in Brazil, reported in New York. Forbes. Available at: https://www.forbes.com/sites/robertglatter/2021/03/21/p1-variant-dominant-strain-in-brazil-reported-in-new-york/?sh=19c9a91e1883 [Accessed April 24, 2021].
89. Hayawi K, Shahriar S, Serhani MA, Alashwal H, Masud MM. **Vaccine versus variants (3Vs): are the COVID-19 vaccines effective against the variants? A systematic review.** *Vaccines* 2021; **9**:1305.
90. Ranzani OT, Hitchings MDT, Dorion M, D'Agostini TL, de Paula RC, de Paula OFP, *et al*. **Effectiveness of the CoronaVac vaccine in older adults during a gamma variant associated epidemic of COVID-19 in Brazil: test negative case-control study.** *BMJ* 2021; **374**:n2015.

91. Hitchings MDT, Ranzani OT, Torres MSS, de Oliveira SB, Almiron M, Said R, *et al*. **Effectiveness of CoronaVac among healthcare workers in the setting of high SARS-CoV-2 Gamma variant transmission in Manaus, Brazil: a test-negative case-control study.** *Lancet Reg Health-Am* 2021; **1**:100025.
92. Skowronski DM, Setayeshgar S, Zou M, Prystajecky N, Tyson JR, Galanis E, *et al*. **Single-dose mRNA vaccine effectiveness against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), including alpha and gamma variants: a test-negative design in adults 70 years and older in British Columbia, Canada.** *Clin Infect Dis* 2021; **1093**:ciab616.
93. COVID-19 vaccine effective against new variant in Brazil. COVID19 vaccine effective against new variant in Brazil. Emerging Pathogens Institute, University of Florida. Available at: https://epi.ufl.edu/articles/covid19-vaccine-effective-against-new-variant-in-brazil.html [Accessed April 24, 2021].
94. Karim SSA, Karim QA. **Omicron SARS-CoV-2 variant: a new chapter in the COVID-19 pandemic.** *Lancet* 2021; **6736**:19–21.
95. Singanayagam A, Hakki S, Dunning J, Madon KJ, Crone MA, Koycheva A, *et al*. **Community transmission and viral load kinetics of the SARS-CoV-2 delta (B.1.617.2) variant in vaccinated and unvaccinated individuals in the UK: a prospective, longitudinal, cohort study.** *Lancet Infect Dis* 2021; **22**:183–195.
96. Cao Y, Wang J, Jian F, Xiao T, Song W, Yisimayi A, *et al*. **Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies.** *Nature* 2021; **602**:657–663.
97. Viana R, Moyo S, Amoako DG, Tegally H, Scheepers C, Althaus CL, *et al*. **Rapid epidemic expansion of the SARS-CoV-2 Omicron variant in southern Africa.** *Nature* 2022; **603**:679–686.
98. GISAID-hCov19 variants. n.d. Available at: https://www.gisaid.org/hcov19-variants/ [Accessed 10 December 2021].
99. Willett BJ, Grove J, MacLean OA, Wilkie C, Logan N, Lorenzo GD, *et al*. **The hyper-transmissible SARS-CoV-2 omicron variant exhibits significant antigenic change, vaccine escape and a switch in cell entry mechanism.** *medRxiv* 2022; **3**:doi: 10.1101/2022.01.03.21268111.
100. Prévost J, Richard J, Gasser R, Ding S, Fage C, Anand SP, *et al*. **Impact of temperature on the affinity of SARS-CoV-2 Spike glycoprotein for host ACE2.** *J Biol Chem* 2021; **297**:101151.
101. Yang TJ, Yu PY, Chang YC, Liang KH, Tso HC, Ho MR, *et al*. **Impacts on the structure-function relationship of SARS-CoV-2 spike by B.1.1.7 mutations.** *bioRxiv* 2021doi: 10.1101/2021.05.11.443686.
102. Wang P, Nair MS, Liu L, Iketani S, Luo Y, Guo Y, *et al*. **Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7.** *Nature* 2021; **593**:130–135.
103. Nishiura H, Ito K, Anzai A, Kobayashi T, Piantham C, Rodríguez-Morales AJ. **Relative reproduction number of SARS-CoV-2 Omicron (B.1.1.529) compared with Delta variant in South Africa.** *J Clin Med* 2021; **11**:30.
104. Wang L, Berger NA, Kaelber DC, Davis PB, Volkow ND, Xu R. **Comparison of outcomes from COVID infection in pediatric and adult patients before and after the emergence of Omicron.** *medRxiv* 2022; **2**:2021.12.30.21268495. doi: 10.1101/2021.12.30.21268495. Preprint.
105. Lewnard JA, Hong VX, Patel MM, Kahn R, Lipsitch M, Tartof SY. **Clinical outcomes associated with SARS-CoV-2 Omicron (B.1.1.529) variant and BA.1/BA.1.1 or BA.2 subvariant infection in Southern California.** *Nature Medicine* 2022; **28**:1933–1943.
106. Pegu A, O'Connell SE, Schmidt SD, O'Dell S, Talana CA, Lai L, *et al*. **Durability of mRNA-1273 vaccine-induced antibodies against SARS-CoV-2 variants.** *Science* 2021; **373**:1372–1377.
107. Garcia-Beltran WF, St Denis KJ, Hoelzemer A, Lam EC, Nitido AD, Sheehan ML, *et al*. **mRNA-based COVID-19 vaccine boosters induce neutralizing immunity against SARS-CoV-2 Omicron variant.** *Cell* 2022; **185**:457–466e4.
108. Lyngse FP, Mortensen LH, Denwood MJ, Christiansen LE, Møller CH, Skov RL, *et al*. **Household transmission of the SARS-CoV-2 Omicron variant in Denmark.** *Nat Commun* 2022; **13**:5573.
109. Bolze A, Basler T, White S, Dei Rossi A, Wyman D, Dai H, *et al*. **Evidence for SARS-CoV-2 Delta and omicron co-infections and recombination.** *Med* 2022; **3**:848–859.e4.
110. Colson P, Fournier PE, Delerce J, Million M, Bedotto M, Houhamdi L, *et al*. **Culture and identification of a "Deltamicron" SARS-CoV-2 in a three cases cluster in southern France.** *J Med Virol* 2022; **94**:3739–3749.
111. Giovanetti M, Benedetti F, Campisi G, Ciccozzi A, Fabris S, Ceccarelli G, *et al*. **Evolution patterns of SARS-CoV-2: snapshot on its genome variants.** *Biochem Biophys Res Commun* 2021; **538**:88–91.
112. Fernandes Q, Inchakalody VP, Merhi M, Mestiri S, Taib N, Moustafa Abo El-Ella D, *et al*. **Emerging COVID-19 variants and their impact on SARS-CoV-2 diagnosis, therapeutics and vaccines.** *Ann Med* 2022; **54**:524–540.
113. Andreano E, Piccini G, Licastro D, Casalino L, Johnson NV, Paciello I, *et al*. **SARS-CoV-2 escape in vitro from a highly neutralizing COVID-19 convalescent plasma.** *bioRxiv* 2020; **28**:doi: 10.1101/2020.12.28.424451.
114. Wang Z, Schmidt F, Weisblum Y, Muecksch F, Barnes CO, Finkin S, *et al*. **mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants.** *Nature* 2021; **592**:616–622.
115. Vasireddy D, Vanaparthy R, Mohan G, Malayala SV, Atluri P. **Review of COVID-19 variants and COVID-19 vaccine efficacy: what the clinician should know?** *J Clin Med Res* 2021; **13**:317–325.
116. Graichen H. **What is the difference between the first and the second/third wave of Covid-19? – German perspective.** *J Orthop* 2021; **24**:A1–A3.
117. Xu S, Li Y. **Beware of the second wave of COVID-19.** *Lancet* 2020; **395**:1321–1322.
118. Chersich MF, Gray G, Fairlie L, Eichbaum Q, Mayhew S, Allwood B, *et al*. **Covid-19 in Africa: care and protection for frontline healthcare workers.** *Global Health* 2020; **16**:46.
119. Soriano V, Ganado-Pinilla P, Sanchez-Santos M, Gómez-Gallego F, Barreiro P, de Mendoza C, Corral O. **Main differences between the first and second waves of COVID-19 in Madrid, Spain.** *Int J Infect Dis* 2021; **105**:374–376.
120. Zhang SX, Arroyo Marioli F, Gao R. **A second wave? What do people mean by COVID waves? a working definition of epidemic waves.** *Res Manag Healthc Policy* 2021; **14**:3775–3782.
121. El-Shabasy RM, Nayel MA, Taher MM, Abdelmonem R, Shoueir KR, Kenawy ER. **Three waves changes, new variant strains, and vaccination effect against COVID-19 pandemic.** *Int J Biol Macromol* 2022; **204**:161–168.
122. Hu J, Li C, Wang S, Li T, Zhang H. **Genetic variants are identified to increase risk of COVID-19 related mortality from UK Biobank data.** *Hum Genomics* 2020; **15**:10.
123. Malik JA, Ahmed S, Mir A, Shinde M, Bender O, Alshammari F, *et al*. **The SARS-CoV-2 mutations versus vaccine effectiveness: New opportunities to new challenges.** *J Infect Public Health* 2022; **15**:228–240.
124. Rypdal K, Bianchi FM, Rypdal M. **Intervention fatigue is the primary cause of strong secondary waves in the COVID-19 pandemic.** *Int J Environ Res Public Health* 2020; **17**:1–17.
125. Thompson HA, Mousa A, Dighe A, Fu H, Arnedo-Pena A, Barrett P, *et al*. **Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) setting-specific transmission rates: a systematic review and meta-analysis.** *Clin Infect Dis* 2021; **73**:e754–e764.
126. Soriano V, Sánchez-Santos M, Barreiro P. **Rapid antigen testing and mask wearing while waiting for COVID-19 vaccines.** *AIDS Rev* 2020; **22**:168–172.
127. Griffin D, Brennan-Rieder D, Ngo B, Kory P, Confalonieri M, Shapiro L, *et al*. **The importance of understanding the stages of COVID-19 in treatment and trials.** *AIDS Rev* 2021; **23**:40–47.
128. Ngo BT, Marik P, Kory P, Shapiro L, Thomadsen R, Iglesias J, *et al*. **The time to offer treatments for COVID-19.** *Expert Opin Investig Drugs* 2021; **30**:505–518.
129. Soriano V, de Mendoza C, Gómez-Gallego F, Corral O, Barreiro P. **Third wave of COVID-19 in Madrid, Spain.** *Int J Infect Dis* 2021; **107**:212–214.
130. Moyle PM, Toth I. **Modern subunit vaccines: development, components, and research opportunities.** *ChemMedChem* 2013; **8**:360–376.
131. Liljeqvist S, Ståhl S. **Production of recombinant subunit vaccines: protein immunogens, live delivery systems and nucleic acid vaccines.** *J Biotechnol* 1999; **73**:1–33.
132. Roy P, Noad R. **Virus-like particles as a vaccine delivery system: myths and facts.** *Hum Vaccin* 2008; **4**:5–12.
133. Wang N, Shang J, Jiang S, Du L. **Subunit vaccines against emerging pathogenic human coronaviruses.** *Front Microbiol* 2020; **11**:298.
134. Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, *et al*. **Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV.** *Nat Commun* 2020; **11**:1620.

135. Jiaming L, Yanfeng Y, Yao D, Yawei H, Linlin B, Baoying H, *et al*. **The recombinant N-terminal domain of spike proteins is a potential vaccine against Middle East respiratory syndrome coronavirus (MERS-CoV) infection.** *Vaccine* 2017; **35**:10–18.
136. Keech C, Albert G, Cho I, Robertson A, Reed P, Neal S, *et al*. **Phase 1-2 trial of a SARS-CoV-2 recombinant spike protein nanoparticle vaccine.** *N Engl J Med* 2020; **383**:2320–2332.
137. Magnusson SE, Altenburg AF, Bengtsson KL, Bosman F, de Vries RD, Rimmelzwaan GF, Stertman L. **Matrix-M^{TM} adjuvant enhances immunogenicity of both protein-and modified vaccinia virus Ankara-based influenza vaccines in mice.** *Immunol Res* 2018; **66**:224–233.
138. Tian JH, Patel N, Haupt R, Zhou H, Weston S, Hammond H, *et al*. **SARS-CoV-2 spike glycoprotein vaccine candidate NVX-CoV2373 immunogenicity in baboons and protection in mice.** *Nat Commun* 2021; **12**:372.
139. Guebre-Xabier M, Patel N, Tian JH, Zhou B, Maciejewski S, Lam K, *et al*. **NVX-CoV2373 vaccine protects cynomolgus macaque upper and lower airways against SARS-CoV-2 challenge.** *Vaccine* 2020; **38**:7892–7896.
140. Wadman M. **The long shot.** *Science* 2020; **370**:649–653.
141. Heath PT, Galiza EP, Baxter DN, Boffito M, Browne D, Burns F, *et al*. **Safety and efficacy of NVX-CoV2373 covid-19 vaccine.** *N Engl J Med* 2021; **385**:1172–1183.
142. Mascellino MT, Di Timoteo F, De Angelis M, Oliva A. **Overview of the main antisars-cov-2 vaccines: mechanism of action, efficacy and safety.** *Infect Drug Resist* 2021; **14**:3459–3476.
143. Novavax vaccine 96% effective against original coronavirus, 86% vs British variant in UK trial | Reuters. n.d. Available at: https://www.reuters.com/article/uk-health-coronavirus-vaccines-novavax-idUSKBN2B32ZI.
144. Ryzhikov AB, Ryzhikov EA, Bogryantseva MP, Usova SV, Nechaeva EA, Pyankov OV, *et al*. **A single blind, placebo-controlled randomized study of the safety, reactogenicity and immunogenicity of the "EpiVacCorona" vaccine for the prevention of COVID-19, in volunteers aged 18-60 years (Phase I-II).** *Russ J Infect Immun* 2021; **11**:283–296.
145. Costanzo M, De Giglio MA, Roviello GN. **Anti-coronavirus vaccines: past investigations on SARS-CoV-1 and MERS-CoV, the approved vaccines from BioNTech/Pfizer, Moderna, Oxford/AstraZeneca and others under Development Against SARSCoV-2 infection.** *Curr Med Chem* 2022; **29**:4–18.
146. Yang S, Li Y, Dai L, Wang J, He P, Li C, *et al*. **Safety and immunogenicity of a recombinant tandem-repeat dimeric RBD-based protein subunit vaccine (ZF2001) against COVID-19 in adults: two randomised, double-blind, placebo-controlled, phase 1 and 2 trials.** *Lancet Infect Dis* 2021; **21**:1107–1119.
147. Zhao X, Zheng A, Li D, Zhang R, Sun H, Wang Q, *et al*. **Neutralisation of ZF2001-elicited antisera to SARS-CoV-2 variants.** *Lancet Microbe* 2021; **2**:e494.
148. Wu Z, Li T. **Nanoparticle-mediated cytoplasmic delivery of messenger RNA vaccines: challenges and future perspectives.** *Pharm Res* 2021; **38**:473–478.
149. Alfagih IM, Aldosari B, Alquadeib B, Almurshedi A, Alfagih MM. **Nanoparticles as adjuvants and nanodelivery systems for mRNA-based vaccines.** *Pharmaceutics* 2021; **13**:1–27.
150. Mendonça SA, Lorincz R, Boucher P, Curiel DT. **Adenoviral vector vaccine platforms in the SARS-CoV-2 pandemic.** *npj Vaccines* 2021; **6**:97.
151. Schlake T, Thess A, Fotin-Mleczek M, Kallen KJ. **Developing mRNA-vaccine technologies.** *RNA Biol* 2012; **9**:1319–1330.
152. Dawar S, Jain M. **Current status of the coronavirus vaccination development: a review.** *Ann Rom Soc Cell Biol* 2021; **25**:16650–16662.
153. Wang F, Kream RM, Stefano GB. **An evidence based perspective on mRNA-SARS-CoV-2 vaccine development.** *Med Sci Monit* 2020; **26**:1–8.
154. Yadav T, Srivastava N, Mishra G, Dhama K, Kumar S, Puri B, *et al*. **Recombinant vaccines for COVID-19.** *Hum Vaccines Immunother* 2020; **16**:2905–2912.
155. Pardi N, Hogan MJ, Porter FW, Weissman D. **mRNA vaccines – a new era in vaccinology.** *Nat Rev Drug Discov* 2018; **17**:261–279.
156. Tan L, Sun X. **Recent advances in mRNA vaccine delivery.** *Nano Res* 2018; **11**:5338–5354.
157. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, *et al*. **Safety and efficacy of the BNT162b2 mRNA covid-19 vaccine.** *N Engl J Med* 2020; **383**:2603–2615.
158. Schoenmaker L, Witzigmann D, Kulkarni JA, Verbeke R, Kersten G, Jiskoot W, *et al*. **mRNA-lipid nanoparticle COVID-19 vaccines: Structure and stability.** *Int J Pharm* 2021; **601**:120586.
159. Abu-Raddad LJ, Chemaitelly H, Butt AA, National Study Group for COVID-19 Vaccination. **Effectiveness of the BNT162b2 COVID-19 vaccine against the B.1.1.7 and B.1.351 variants.** *N Engl J Med* 2021; **385**:187–189.
160. Hall VJ, Foulkes S, Saei A, Andrews N, Oguti B, Charlett A, *et al*. **COVID-19 vaccine coverage in health-care workers in England and effectiveness of BNT162b2 mRNA vaccine against infection (SIREN): a prospective, multicentre, cohort study.** *Lancet* 2021; **397**:1725–1735.
161. Lopez Bernal J, Andrews N, Gower C, Gallagher E, Simmons R, Thelwall S, *et al*. **Effectiveness of covid-19 vaccines against the B.1.617.2 (Delta) variant.** *N Engl J Med* 2021; **385**:585–594.
162. Sheikh A, Robertson C, Taylor B. **BNT162b2 and ChAdOx1 Ncov-19 vaccine effectiveness against death from the delta variant.** *N Engl J Med* 2021; **385**:2195–2197.
163. Parry H, Tut G, Faustini S, Bruton R, Stephens C, Saunders P, *et al*. **mRNA vaccination in people over 80 years of age induces strong humoral immune responses against SARS-CoV-2 with cross neutralization of P.1 Brazilian variant.** *Elife* 2021; **10**:e69375.
164. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, *et al*. **Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine.** *N Engl J Med* 2021; **384**:403–416.
165. Martínez-Baz I, Trobajo-Sanmartín C, Miqueleiz A, Guevara M, Fernández-Huerta M, Burgui C, *et al*. **Product-specific COVID-19 vaccine effectiveness against secondary infection in close contacts, Navarre, Spain, April to August 2021.** *Euro Surveill* 2021; **26**:2100894.
166. Boloko L, Lifson A, Little F, De Wet T, Papavarnavas N, Marais G, *et al*. **Severity and inpatient mortality of COVID-19 pneumonia from beta variant infection: a clinical cohort study in Cape Town, South Africa.** *medRxiv* 2021; **4**:2021.11.04.21265916. doi: 10.1101/2021.11.04.21265916. PPR: PPR416318.
167. Bruxvoort KJ, Sy LS, Qian L, Ackerson BK, Luo Y, Lee GS, *et al*. **Effectiveness of mRNA-1273 against delta, mu, and other emerging variants of SARS-CoV-2: Test Negative Case-Control Study.** *BMJ* 2021; **375**:e068848.
168. Šmíd M, Berec L, Přibylová L, Májek O, Pavlík T, Jarkovský J, *et al*. **Protection by vaccines and previous infection against the omicron variant of SARS-CoV-2.** *J Infect Dis* 2022; **226**:1385–1390.
169. Kremsner PG, Mann P, Kroidl A, Leroux-Roels I, Schindler C, Gabor JJ, *et al*. **Safety and immunogenicity of an mRNA-lipid nanoparticle vaccine candidate against SARS-CoV-2.** *Wien Klin Wochenschr* 2021; **133**:931–941.
170. Kremsner PG, Ahuad Guerrero RA, Arana-Arri E, Aroca Martinez GJ, Bonten M, Chandler R, *et al*. **Efficacy and safety of the CVnCoV SARS-CoV-2 mRNA vaccine candidate in ten countries in Europe and Latin America (HERALD): a randomised, observer-blinded, placebo-controlled, phase 2b/3 trial.** *Lancet Infect Dis* 2022; **22**:329–340.
171. Lee LYY, Izzard L, Hurt AC. **A review of DNA vaccines against influenza.** *Front Immunol* 2018; **9**:1568.
172. Li L, Petrovsky N. **Molecular mechanisms for enhanced DNA vaccine immunogenicity.** *Expert Rev Vaccines* 2016; **15**:313–329.
173. Dupuis M, Denis-Mize K, Woo C, Goldbeck C, Selby MJ, Chen M, *et al*. **Distribution of DNA vaccines determines their immunogenicity after intramuscular injection in mice.** *J Immunol* 2000; **165**:2850–2858.
174. Kaur SP, Gupta V. **COVID-19 vaccine: a comprehensive status report.** *Virus Res* 2020; **288**:198114.
175. Inovio Accelerates Timeline for COVID-19 DNA Vaccine INO-4800, can be found under http://ir.inovio.com/news-and-media/news/press-release-details/2020/Inovio-Accelerates-Timeline-for-COVID-19-DNA-Vaccine-INO-4800/default.aspx.
176. INOVIO Expands manufacturing of COVID-19 DNA vaccine INO-4800 with new funding from CEPI. Available at: http://ir.inovio.com/news-releases/news-releases-details/2020/INOVIO-Expands-Manufacturing of-COVID-19-DNA-Vaccine-INO-4800-With-New-Fundingfrom-CEPI/default.aspx [cited 2020 May 18].
177. Smith TRF, Patel A, Ramos S, Elwood D, Zhu X, Yan J, *et al*. *Nat Commun* 2020; **11**:2601.

178. Safety and Immunogenicity of INO-4800, a COVID-19 DNA vaccine as a primary series and booster. Available at: https://www.researchgate.net/publication/356795013_571_Safety_and_Immunogenicity_of_INO-4800_a_COVID.
179. Zydus receives EUA from DCGI for ZyCoV-D, the only needle-free COVID vaccine in the world. Accessed September 2, 2021.
180. Zycov-D, India's first COVID-19 vaccine for those above 12, gets nod. The Hindu. 2021. Accessed September 2, 2021.
181. Dey A, Chozhavel Rajanathan TM, Chandra H, Pericherla HRR, Kumar S, Choonia HS, *et al*. **Immunogenic potential of DNA vaccine candidate, ZyCoV-D against SARS-CoV-2 in animal models.** *Vaccine* 2021; **39**:4108–4116.
182. Yadav PD, Kumar S, Agarwal K, Jain MR, Patil DR, Maithal K, *et al*. **Assessment of immunogenicity and protective efficacy of ZyCoV-D DNA vaccine candidates in Rhesus macaques against SARS-CoV-2 infection.** *bioRxiv* 2021doi: 10.1101/2021.02.02.429480.
183. Momin T, Kansagra K, Patel H, Sharma S, Sharma B, Patel J, *et al*. **Safety and immunogenicity of a DNA SARS-CoV-2 vaccine (ZyCoV-D): results of an open-label, nonrandomized phase I part of phase I/II clinical study by intradermal route in healthy subjects in India-NC-ND license.** *EClinicalMedicine* 2021; **38**:101020.
184. Keshavarz M, Mirzaei H, Salemi M, Momeni F, Mousavi MJ, Sadeghalvad M, *et al*. **Influenza vaccine: where are we and where do we go?** *Rev Med Virol* 2019; **29**:e2014.
185. Bandyopadhyay AS, Garon J, Seib K, Orenstein WA. **Polio vaccination: past, present and future.** *Future Microbiol* 2015; **10**:791–808.
186. Kumar A, Meldgaard TS, Bertholet S. **Novel platforms for the development of a universal influenza vaccine.** *Front Immunol* 2018; **9**:600.
187. World Health Organization .Draft landscape and tracker of COVID-19 candidate vaccines; 2021. Available at: https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines.
188. Zhang Y, Zeng G, Pan H. **Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine in healthy adults aged 18-59 years: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial.** *Lancet Infect Dis* 2021; **21**:181–192.
189. Hitchings M, Ranzani OT, Torres MS, de Oliveira SB, Almiron M, Said R, *et al*. **Effectiveness of CoronaVac among healthcare workers in the setting of high SARS-CoV-2 Gamma variant transmission in Manaus, Brazil: a test-negative case-control study.** *Lancet Reg Health Am* 2021; **1**:100025.
190. Center SM. Expert reaction to announcement of results of phase III of Sinovac vaccine in China 2021. Available at: https://www.sciencemediacentre.org/expert-reaction-to-announcement-of-results-of-phase-iii-of-sinovac-vaccine-in-china.
191. Tavsan S. Sinovac's COVID-19 shot is 83% effective, not 91%, turkey says. Nikkei Asia. Available online: https://asia.nikkei.com/Spotlight/Coronavirus/COVID-vaccines/Sinovac-s-COVID-19-shot-is-83-effective-not-91-Turkey-says [Accessed 9 April 2021].
192. Nupus H. Indonesia Approves China's CoronaVac for Emergency Use. 2020. Available at: https://www.aa.com.tr/en/asia-pacific/indonesia-approves-china-s-coronavac-for-emergency-use/2105881 [Accessed 9 April 2021].
193. News Reuters UPDATE 2-China's Sinovac Defends COVID-19 vaccine after disappointing Brazil data. Available at: https://www.reuters.com/article/health-coronavirus-malaysia-idCNL1N2JO0O9 [Accessed 18 March 2021].
194. Wang GL, Wang ZY, Duan LJ, Meng QC, Jiang MD, Cao J, *et al*. **Susceptibility of circulating SARS-CoV-2 variants to neutralization.** *N Engl J Med* 2021; **384**:2354–2356.
195. Chen Y, Shen H, Huang R, Tong X, Wu C. **Serum neutralising activity against SARS-CoV-2 variants elicited by CoronaVac.** *Lancet Infect Dis* 2021; **21**:1071–1072.
196. Amorim MR, Toledo Teixeira DA, De Souza GF, Sesti-Costa R, Coimbra LD, Souza WM, *et al*. **Neutralisation of SARS-CoV-2 lineage P.1 by antibodies elicited through natural SARS-CoV-2 infection or vaccination with an inactivated SARS-CoV-2 vaccine: an immunological study.** *Lancet Microbe* 2021; **2**:e527–e535.
197. Li XN, Huang Y, Wang W, Jing QL, Zhang CH, Qin PZ, *et al*. **Effectiveness of inactivated SARS-CoV-2 vaccines against the Delta variant infection in Guangzhou: a test-negative case-control real-world study.** *Emerg Microbes Infect* 2021; **10**:1751–1759.
198. Araos R, Jara A, Undurragav E, Zubizarreta J, Gonzalez C, Acevedo J, *et al*. **Effectiveness of CoronaVac in children 3 to 5 years during the omicron SARS-CoV-2 outbreak.** *Res Square* 2022; **15**:1–11.
199. Arregoces L, Fernández-Niño J, Rojas-Botero M, Palacios-Clavijo AF, Galvis M, Rincón L, *et al*. **Effectiveness of COVID-19 vaccines in preventing hospitalizations and deaths in Colombia: a pair-matched, national-wide cohort study in older adults.** *SSRN Electronic J* 2021; **19**:1–13.
200. Ella R, Vadrevu KM, Jogdand H, Prasad S, Reddy S, Sarangi V, *et al*. **Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBV152: a double-blind, randomised, phase 1 trial.** *Lancet Infect Dis* 2021; **21**:637–646.
201. Ella R, Reddy S, Jogdand H, Sarangi V, Ganneru B, Prasad S, *et al*. **Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBV152: Interim results from a double-blind, randomised, multicentre, phase 2 trial, and 3-month follow-up of a double-blind, randomised phase 1 trial.** *Lancet Infect Dis* 2021; **21**:950–961.
202. Sapkal GN, Yadav PD, Ella R, Deshpande GR, Sahay RR, Gupta N, *et al*. **Inactivated COVID-19 vaccine BBV152/COVAXIN effectively neutralizes recently emerged B.11.7 variant of SARS-CoV-2.** *J Travel Med* 2021:28doi: 10.1093/jtm/taab051.
203. Yadav PD, Sapkal GN, Ella R, Sahay RR, Nyayanit DA, Patil DY, *et al*. **Neutralization against B.1.351 and B.1.617.2 with sera of COVID-19 recovered cases and vaccinees of BBV152.** *bioRxiv* 2021doi: 10.1101/2021.06.05.447177. 2021.06.05.447177.
204. Yadav PD, Sapkal GN, Abraham P, Ella R, Deshpande G, Patil DY, *et al*. **Neutralization of variant under investigation B.1.617 with sera of BBV152 vaccinees.** *Clin Infect Dis* 2021:ciab411doi: 10.1093/cid/ciab411.
205. Bharat Biotech's Covaxin set for trials on children aged between 2 to 18 years. Available at: https://www.india.com/news/india/coronavirus-vaccine-news-today-12-may-2021-bharat-biotech-covaxin-clinical-trials-on-children-between-2-to-18-years-age-group-4657989/.
206. Ella R, Reddy S, Blackwelder W, Potdar V, Yadav P, Sarangi V, *et al*. **Efficacy, safety, and lot-to-lot immunogenicity of an inactivated SARS-CoV-2 vaccine (BBV152): interim results of a randomised, double-blind, controlled, phase 3 trial.** *Lancet* 2021; **398**:2173–2184.
207. Xia S, Zhang Y, Wang Y, Wang H, Yang Y, Gao GF, *et al*. **Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomised, double-blind, placebo-controlled, phase 1/2 trial.** *Lancet Infect Dis* 2021; **21**:39–51.
208. Immunization ON. Evidence assessment: Sinopharm/BBIB COVID-19 vaccine. n.d.
209. UAE: Ministry of Health announces 86 per cent vaccine efficacy. 9 December 2020. Available at: https://gulfnews.com/uae/health/uae-ministry-of-health-announces-86-per-cent-vaccine-efficacy-1.1607490555571.].
210. News A. Coronavirus: Morocco receives half million doses of Sinopharm COVID-19 vaccine 2021-01-28. Available at: https://english.alarabiya.net/coronavirus/2021/01/27/Coronavirus-Coronavirus-Morocco-receives-half-million-doses-of-Sinopharm-COVID-19-vaccine.
211. GLOBALink. Algeria receives donation of 200,000 doses of anti-COVID-19 vaccine from China. 25 February, 2021. Available at: http://www.xinhuanet.com/english/2021-02/25/c_139766759.htm.
212. News U. Serbia receives million doses of China's Sinopharm COVID-19 vaccine. January 16, 2021. Available at: https://www.usnews.com/news/world/articles/2021-01-16/serbia-receives-million-doses-of-chinas-sinopharm-covid-19-vaccine.
213. Le Tung Thanh, Andreadakis Zacharias, Kumar Arun, Román Raúl Gómez, Tollefsen Stig, Saville Melanie, Mayhew Stephen. The COVID-19 vaccine development landscape. Nat Rev Drug Discovery. 2020;19:305–306.
214. Francis AI, Ghany S, Gilkes T, Umakanthan S. **Review of COVID-19 vaccine subtypes, efficacy and geographical distributions.** *Postgrad Med J* 2021; **98**:389–394.
215. Teo SP. **Review of covid-19 vaccines and their evidence in older adults.** *Ann Geriatr Med Res* 2021; **25**:4–9.
216. Creech CB, Walker SC, Samuels RJ. **SARS-CoV-2 vaccines.** *JAMA* 2021; **325**:1318–1320.
217. Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-Rammerstorfer S, *et al*. **Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial.** *Lancet* 2020; **396**:467–478.

218. Tracker CV. COVID-19 vaccine tracker: Oxford/AstraZeneca vaccine; 2021. Available at: https://covid19.trackvaccines.org/vaccines/4/.
219. Voysey M, Clemens SAC, Madhi SA, Weckx LY, Folegatti PM, Aley PK, *et al*. **Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK.** *Lancet* 2021; **397**:99–111.
220. Voysey M, Costa Clemens SA, Madhi SA, Weckx LY, Folegatti PM, Aley PK, *et al*. **Single-dose administration and the influence of the timing of the booster dose on immunogenicity and efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine: a pooled analysis of four randomised trials.** *Lancet* 2021; **397**:881–891.
221. Lopez Bernal J, Andrews N, Gower C, Gallagher E, Simmons R, Thelwall S, *et al*. **Effectiveness of COVID-19 vaccines against the B.1.617.2 (Delta) variant.** *N Engl J Med* 2021; **385**:585–594.
222. Sheikh A, McMenamin J, Taylor B, Robertson C. **Public Health Scotland and the EAVE II Collaborators. SARS-CoV-2 Delta VOC in Scotland: demographics, risk of hospital admission, and vaccine effectiveness.** *Lancet* 2021; **397**:2461–2462.
223. Emary KRW, Golubchik T, Aley PK, Ariani CV, Angus B, Bibi S, *et al*. **Efficacy of ChAdOx1 Ncov-19 (AZD1222) vaccine against SARS-CoV-2 variant of concern 202012/01 (B.1.1.7): an exploratory analysis of a randomised controlled trial.** *Lancet* 2021; **397**:1351–1362.
224. Marra AR, Miraglia JL, Malheiros DT, Guozhang Y, Teich VD, da SilvaVictor E, *et al*. **Effectiveness of two coronavirus disease 2019 (COVID-19) vaccines (viral vector and inactivated viral vaccine) against severe acute respiratory coronavirus virus 2 (SARS-CoV-2) infection in a cohort of healthcare workers.** *Infect Control Hosp Epidemiol* 2022; **44**:75–81.
225. Hitchings MDT, Ranzani OT, Dorion M, D'Agostini TL, de Paula RC, de Paula OFP, *et al*. **Effectiveness of the ChAdOx1 vaccine in the older adults during SARS-CoV-2 Gamma variant circulation in Sao Paulol.** *Nat Commun* 2021; **12**:6220.
226. Šmíd M, Berec L, Přibylová L, Májek O, Pavlík T, Jarkovský J, *et al*. **Protection by vaccines and previous infection against the omicron variant of severe acute respiratory syndrome coronavirus 2.** *J Infect Dis* 2022; **226**:1385–1390.
227. Logunov DY, Dolzhikova IV, Zubkova OV, Tukhvatullin AI, Shcheblyakov DV, Dzharullaeva AS, *et al*. **Safety and immunogenicity of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine in two formulations: two open, nonrandomised phase 1/2 studies from Russia.** *Lancet* 2020; **396**:887–897.
228. Logunov DY, Dolzhikova IV, Shcheblyakov DV, Tukhvatulin AI, Zubkova OV, Dzharullaeva AS, *et al*. **Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia.** *Lancet* 2021; **397**:671–681.
229. Ikegame S, Siddiquey MNA, Hung CT, Haas G, Brambilla L, Oguntuyo KY, *et al*. **Neutralizing activity of Sputnik V vaccine sera against SARS-CoV-2 variants.** *Nat Commun* 2021; **12**:4598.
230. Russia's Sputnik V shot around 90% effective against Delta variant, developers say. Reuters. Accessed September 2, 2021.
231. Anadolu Agency Decision Based on Results of Country's Phase III Trials That Put Estimated Vaccine Efficacy at 65.3%, Says Official. Available at: https://www.astrazeneca.com/media-centre/press-releases/2021/azd1222-us-phase-iii-primary-analysis-confirms-safety-and-efficacy.html [Accessed 18 March 2021].
232. Mercado NB, Zahn R, Wegmann F. **Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques.** *Nature* 2020; **586**:583–588.
233. Stephenson KE, Le Gars M, Sadoff J, de Groot AM, Heerwegh D, Truyers C, *et al*. **Immunogenicity of the Ad26.COV2.S vaccine for COVID-19.** *JAMA* 2021; **325**:1535–1544.
234. Sadoff J, Gray G, Vandebosch A, Cárdenas V, Shukarev G, Grinsztejn B, *et al*. **Safety and efficacy of single-dose Ad26.COV2.S vaccine against covid-19.** *N Engl J Med* 2021; **384**:2187–2201.
235. Abdool Karim SS, de Oliveira T. **New SARS-CoV-2 variants-clinical, public health, and vaccine implications.** *N Engl J Med* 2021; **384**:1866–1868.
236. Ranzani OT, Rogério L, Castilho LD, Gonçalves C, Resende G, Rosana M, *et al*. **Vaccine effectiveness of Ad26.COV2.S against symptomatic COVID-19 and clinical outcomes in Brazil: a test-negative study design.** *medRxiv* 2021; **15**:2021.10.15.21265006. doi: 10.1101/2021.10.15.21265006.
237. It's not just Johnson & Johnson: China has a single-dose COVID-19 vaccine that has 65% efficacy. Fortune. Retrieved 2021-02-11.
238. Zhu FC, Li YH, Guan XH, Hou LH, Wang WJ, Li JX, *et al*. **Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, nonrandomised, first-in-human trial.** *Lancet* 2020; **395**:1845–1854.
239. China's single-dose COVID vaccine has efficacy similar to Johnson & Johnson's. Fortune. Accessed September 2, 2021.
240. Minor PD. **Live attenuated vaccines: historical successes and current challenges.** *Virology* 2015; **479**:379–392.
241. Graham RL, Donaldson EF, Baric RS. **A decade after SARS: strategies for controlling emerging coronaviruses.** *Nat Rev Microbiol* 2013; **11**:836–848.
242. Wang Y, Yang C, Song Y, Coleman JR, Stawowczyk M, Tafrova J, *et al*. **Scalable live-attenuated SARS-CoV-2 vaccine candidate demonstrates preclinical safety and efficacy.** *Proc Natl Acad Sci USA* 2021; **118**:1–7.
243. Anon. The University of Hong Kong; 2020. HKU joins global partnership to develo COVID-19 vaccine. Available at: https://fightcovid19.hku.hk/hku-state-key-laboratory-for-emerging-infectious-diseases-joins-global-effort-to-develop-covid-19-vaccine/https://fightcovid19.hku.hk/. [Accessed March 18, 2020].
244. Mohsen MO, Augusto G, Bachmann MF. **The 3Ds in virus-like particle based-vaccines: "design, delivery and dynamics".** *Immunol Rev* 2020; **296**:155–168.
245. Li YD, Chi WY, Su JH, Ferrall L, Hung CF, Wu TC. **Coronavirus vaccine development: from SARS and MERS to COVID-19.** *J Biomed Sci* 2020; **27**:1–23.
246. Philippidis A. **COVID-19: top 60 drug treatments in development: the biopharma industry is ramping up the development of dozens of potential drug therapies and clinical testing in an all-hands effort to combat the pandemic.** *Genet Eng Biotechnol News* 2020; **40**:10–13.
247. Kushnir N, Streatfield SJ, Yusibov V. **Virus-like particles as a highly efficient vaccine platform: diversity of targets and production systems and advances in clinical development.** *Vaccine* 2012; **31**:58–83.
248. Cubas R, Zhang S, Kwon S, Sevick-Muraca EM, Li M, Chen C, Yao Q. **Virus-like particle (VLP) lymphatic trafficking and immune response generation after immunization by different routes.** *J Immunother* 2009; **32**:118–128.
249. Mohsen M, Gomes A, Vogel M, Bachmann M. **Interaction of viral capsid-derived virus-like particles (VLPs) with the innate immune system.** *Vaccines* 2018; **6**:37.
250. Win SJ, Ward VK, Dunbar PR, Young SL, Baird MA. **Cross-presentation of epitopes on virus-like particles via the MHC I receptor recycling pathway.** *Immunol Cell Biol* 2011; **89**:681–688.
251. Tan TK, Rijal P, Rahikainen R, Keeble AH, Schimanski L, Hussain S, *et al*. **A COVID-19 vaccine candidate using SpyCatcher multimerization of the SARS-CoV-2 spike protein receptor-binding domain induces potent neutralising antibody responses.** *Nat Commun* 2021; **12**:1–16.
252. Plescia CB, David EA, Patra D, Sengupta R, Amiar S, Su Y, *et al*. **SARS-CoV-2 viral budding and entry can be modeled using BSL-2 level virus-like particles.** *J Biol Chem* 2021; **296**:100103.
253. Khan WH, Hashmi Z, Goel A, Ahmad R, Gupta K, Khan N, *et al*. **COVID-19 pandemic and vaccines update on challenges and resolutions.** *Front Cell Infect Microbiol* 2021; **11**:690621.
254. Expert Meeting on the Novel Coronavirus Disease Control. Analysis of the response to the novel coronavirus (COVID-19) and recommendations. March 19, 2020.
255. Preventing the spread of the new coronavirus infection through cluster countermeasures. Ministry of Health, Labour and Welfare. 25 February 2020 (PDF in Japanese).
256. Battling the coronavirus: an interview with Professor Kawaoka Yoshihiro. NHK WORLD-JAPAN News. NHK WORLD. Retrieved 12 May 2020.
257. Japan to downgrade coronavirus classification on May 8, 2023. Jap Med J. Retrieved 11 February 2023.
258. Evaluation of the latest infection status of the new coronavirus infection, etc. 124th (August 4, 2023) New Coronavirus Infectious Disease Countermeasures Advisory Board.
259. Birkhoff M, Leitz M, Marx D. **Advantages of intranasal vaccination and considerations on device selection.** *Indian J Pharm Sci* 2009; **71**:729–731.

260. Li Y, Jin L, Chen T. **The effects of secretory IgA in the mucosal immune system.** *Biomed Res Int* 2020; **2020**:2032057.
261. Gallo O, Locatello LG, Mazzoni A, Novelli L, Annunziato F. **The central role of the nasal microenvironment in the transmission, modulation, and clinical progression of SARS-CoV-2 infection.** *Mucosal Immunol* 2021; **14**:305–316.
262. Alu A, Chen L, Lei H, Wei Y, Tian X, Wei X. **Intranasal COVID-19 vaccines: from bench to bed.** *EBioMedicine* 2022; **76**:103841.
263. Wang S, Liu H, Zhang X, Qian F. **Intranasal and oral vaccination with protein-based antigens: advantages, challenges and formulation strategies.** *Protein Cell* 2015; **6**:480–503.
264. Reboldi A, Cyster JG. **Peyer's patches: organizing B-cell responses at the intestinal frontier.** *Immunol Rev* 2016; **271**:230–245.
265. Boyaka PN. **Inducing mucosal IgA: a challenge for vaccine adjuvants and delivery systems.** *J Immunol* 2017; **199**:9–16.
266. Quraish RU, Hirahata T, Quraish AU, Ul Quraish S. **An overview: genetic tumor markers for early detection and current gene therapy strategies.** *Cancer Inform* 2023; **22**:11769351221150772.
267. Janssen Ad26.COV2.S in Proceedings of the vaccine for the prevention of COVID-19; 26 February 2021. Available at: https://www.fda.gov/advisory-committees/advisory-committeecalendar/vaccines-and-related-biological-products-advisory-committee-february-26-2021-meeting-announcement [Accessed 25 March 2021].
268. Bongiovanni M. **COVID-19 reinfection in a healthcare worker.** *J Med Virol* 2020; **93**:4058–4059.
269. To KK, Hung IF, Ip JD, Chu AW, Chan WM, Tam AR, *et al*. **Coronavirus disease 2019 (COVID-19) re-infection by a phylogenetically distinct severe acute respiratory syndrome coronavirus 2 strain confirmed by whole genome sequencing.** *Clin Infect Dis* 2021; **73**:e2946–e2951.
270. Anderson EJ, Rouphael NG, Widge AT, Jackson LA, Roberts PC, Makhene M, *et al*. **Safety and immunogenicity of SARS-CoV-2 mRNA-1273 vaccine in older adults.** *N Engl J Med* 2020; **383**:2427–2438.
271. van Riel D, de Wit E. **Next-generation vaccine platforms for COVID-19.** *Nat Mater* 2020; **19**:810–812.
272. Haun BK, Lai CY, Williams CA, Wong TAS, Lieberman MM, Pessaint L, *et al*. **CoVaccine HT™ adjuvant potentiates robust immune responses to recombinant SARS-CoV-2 spike S1 immunization.** *Front Immunol* 2020; **11**:599587.
273. Phan LT, Nguyen TV, Luong QC, Nguyen TV, Nguyen HT, Le HQ, *et al*. **Importation and human-to-human transmission of a novel coronavirus in Vietnam.** *N Engl J Med* 2020; **382**:872–874.
274. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, *et al*. **Clinical characteristics of 138 hospitalized patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China.** *JAMA* 2020; **323**:1061–1069.
275. Rzymski P, Camargo CA, Fal A, Flisiak R, Gwenzi W, Kelishadi R, *et al*. **COVID-19 vaccine boosters: the good, the bad, and the ugly.** *Vaccines* 2021; **9**:1299.
276. Taylor A, Foo SS, Bruzzone R, Vu Dinh L, King NJC, Mahalingam S, *et al*. **Fc receptors in antibody-dependent enhancement of viral infections.** *Immunol Rev* 2015; **268**:340–364.
277. Wan Y, Shang J, Sun S, Tai W, Chen J, Geng Q, *et al*. **Molecular mechanism for antibody-dependent enhancement of coronavirus entry.** *J Virol* 2020; **94**:e02015–19.
278. Negro F. **Is antibody-dependent enhancement playing a role in COVID-19 pathogenesis?** *Swiss Med Wkly* 2020; **150**:w20249.
279. Lee WS, Wheatley AK, Kent SJ, DeKosky BJ. **Antibody-dependent enhancement and SARS-CoV-2 vaccines and therapies.** *Nat Microbiol* 2020; **5**:1185–1191.
280. Bregu M, Draper SJ, Hill AV, Greenwood BM. **Accelerating vaccine development and deployment: report of a Royal Society satellite meeting.** *Philos Trans R Soc Lond B Biol Sci* 2011; **366**:2841–2849.
281. Gouglas D, Thanh Le T, Henderson K, Kaloudis A, Danielsen T, Hammersland NC, *et al*. **Estimating the cost of vaccine development against epidemic infectious diseases: a cost minimisation study.** *Lancet Glob Health* 2018; **6**:e1386–e1396.
282. Chakraborty C, Sharma AR, Sharma G, Bhattacharya M, Saha RP, Lee SS. **Extensive partnership, collaboration, and teamwork is required to stop the COVID-19 outbreak.** *Arch Med Res* 2020; **51**:728–730.
283. Zenuz AT, Eslami H, Kafil HS, Safari EMA. **The application of antimicrobial photodynamic therapy on *Pseudomonas aeuroginosa* and Enterococcus fecalis using hypericin and methylene blue photosensitizers.** *Biomed Pharmacol J* 2016; **9**:443–450.
284. Vasireddy D, Yusi D, Berrak SG, Lichtenberger J. **Factors affecting refusal rates of the birth dose of hepatitis B vaccine: a single center study.** *J Pediatr Infect* 2014; **8**:159–164.
285. Guidry JPD, Laestadius LI, Vraga EK, Miller CA, Perrin PB, Burton CW, *et al*. **Willingness to get the COVID-19 vaccine with and without emergency use authorization.** *Am J Infect Control* 2021; **49**:137–142.
286. Krammer F. **SARS-CoV-2 vaccines in development.** *Nature* 2020; **586**:516–527.
287. Loomba S, de Figueiredo A, Piatek SJ, de Graaf K, Larson HJ. **Measuring the impact of COVID-19 vaccine misinformation on vaccination intent in the UK and USA.** *Nat Hum Behav* 2021; **5**:337–348.
288. Barach P, Fisher SD, Adams MJ, Burstein GR, Brophy PD, Kuo DZ, Lipshultz SE. **Disruption of healthcare: will the COVID pandemic worsen non-COVID outcomes and disease outbreaks?** *Prog Pediatr Cardiol* 2020; **59**:101254.
289. Chohan UW. **Coronavirus & vaccine nationalism.** *CASS Work Pap Econ Natl Aff* 2021; **2021**:9.
290. Saeed U, Sherdil K, Ashraf U, Younas I, Butt H, Ahmad S. **Identification of potential lockdown areas during COVID-19 transmission in Punjab, Pakistan.** *Public Health* 2021; **190**:42–51.
291. Hussain Y, Muhammad K, Umer MF, Omarkhail A, Khan S, Kamran M, *et al*. **Coronavirus disease 2019 in 5 neighboring limited-resource countries: a financial and health threat.** *Value Health Reg Issues* 2021; **24**:114–116.
292. Fair allocation mechanism for COVID-19 vaccines through the COVAX Facility. Available at: https://www.who.int/publications/m/item/fair-allocation-mechanism-for-covid-19-vaccines-through-the-covax-facility [Accessed April 27, 2021].
293. Kupferschmidt K. **Despite obstacles, WHO unveils plan to distribute vaccine.** *Science* 2020; **369**:1553.
294. Mathieu E, Ritchie H, Ortiz-Ospina E, Roser M, Hasell J, Appel C, *et al*. **A global database of COVID-19 vaccinations.** *Nat Hum Behav* 2021; **5**:947–953.
295. Harvey WT, Carabelli AM, Jackson B, Gupta RK, Thomson EC, Harrison EM, *et al*. **SARS-CoV-2 variants, spike mutations and immune escape.** *Nat Rev Microbiol* 2021; **19**:409–424.