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COVID-19: Clinical status of vaccine development till date

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Abstract

Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) induced COVID-19 is a complicated disease. Clinicians are continuously facing difficulties to treat infected patients using the principle of repurposing of drugs as no specific drugs are available to treat COVID-19. To minimize the severity and casualty, global vaccination is the only hope as a potential preventive measure. After a year-long global research and clinical struggle 165 vaccine candidates are developed and some are in the pipeline till date. Out of them 28 candidate vaccines are approved for their use and rest of them are in the different phases of clinical trials. In this comprehensive report, authors aim to demonstrate, classify, and provide up-to-date clinical trial status of all the vaccines discovered till date and specifically focus onto the approved candidates. At last, specifically focused on the vaccination of different type of medically distinct populations.

Keywords: COVID-19/SARS-CoV-2, Vaccination, Clinical research and vaccine efficacy, Production, Immune responses

1. Introduction

COVID-19 escalated into a global crisis within the initial few months. It has rapidly spread in many countries through human-to-human transmission and was declared as a pandemic disease on March 11, 2020 by World Health Organization (WHO) [1]. Many critical patients require intensive care, immediate use of mechanical ventilation, and reached without getting any chance of urgent medical care [2]. As of December-2021, globally ~272 M confirmed cases and ~5.5 M deaths were reported [3]. Achieving overall victory in this invisible 'world-war' against the COVID-19 and preventing disease spread and recurrence is one of the major challenges for clinicians, research scientists, and leaders of several nations throughout the globe [4,5].

Coronaviruses are mainly classified into four groups *viz.* alpha, beta, gamma, and delta. Among which alpha and beta coronaviruses are of mammal (specifically bats) origin whereas gamma and delta variants emerged from birds and swine, respectively. People infected with Coronaviruses have a potential risk and the virus evolve significantly with continuous mutations.

Coronavirus variants like MERS-CoV and SARS-CoV-1 were studied to have mortality rate of around > 30% and 10-15% respectively, in the infected hosts [6–8]. Throughout the world, pharmaceutical industries and research institutes are working collaboratively to develop a potential preventive strategy. The development of COVID-19 vaccine is an important step to control infection and minimize community spread. Till now, more than a hundred vaccine candidates are in the clinical developmental stages. Few of them, are approved by regulatory bodies of specific countries for their use and still a lot of other vaccine candidates are in different stages of clinical trial [9,10]. Here, we have compiled major information regarding all the approved vaccines, candidate vaccines currently in different phases of the clinical trial and lastly discussed about the vaccination of medically distinct groups.

2. Classification, development, and clinical status of COVID-19 vaccines

The frequent mutational changes in the viral structure make it very difficult to treat them due to the less or non-availability of specific anti-SARS-CoV-2 therapeutic agents and change in drug targets. Generally, the antiviral therapeutic approach is "one bug - one drug" and often it's no drugs. Therefore, vaccines are the most valuable approach against COVID-19 and need to be taken in advance by the host for immunization before infection. It will take time and huge effort for development and validation of vaccines through preclinical and clinical trials [11,12]. Here, we have divided the vaccines into two major classes a) component vaccines and b) whole virus vaccines and discuss their clinical development in detail (Fig. 1).

2.1. Component vaccines

As the name indicates, component vaccines are those which utilize the individual components of viral particles to train our immune system and prepare it in case of future viral exposure and keep us safe and healthy [13]. The component vaccines are classified into six types and are discussed below.

2.1.1. DNA vaccines

DNA vaccines contain genetic material of pathogens that encodes viral antigenic response expressed from plasmid vectors and finally delivered into the host. It is a precise and flexible strategy to deliver the antigens/plasmid DNAs and additional components through electroporation. After successful inoculation, plasmid DNA based vaccines are capable of inducing T cell activation which may induce neutralizing antibodies. Recently, a research group has developed a series of DNA vaccines that induce antibodies against different components of S protein. In animal studies, these vaccines were documented to induce both humoral as well as cellular immunity; with high titer of neutralizing antibodies [14–17]. It is noticed that different kind of vaccine candidates are specifically targeted or designed against specific viral epitopes *viz.*, spike proteins (S1, S2, and S2'), receptor binding domains, nucleocapsid (N), and membrane (M) proteins that produces specific immunological responses (Table 1) [18].

Production — They are produced by the incorporation of antigen containing eukaryotic expression vector into bacterial system. The elements of plasmid backbone help in the propagation and selection of the positive vector colonies in bacteria. Generally, *E. coli* is preferred for the replication because that gives high copy number of a plasmid in the presence of kanamycin as a selectable marker. In the new of era of vaccines, for human use, regulatory bodies replace the antibiotic resistance markers present in the plasmid backbone [19]. Additionally, the DNA constructs (semi-synthetic minicircle DNA [20] and fully synthetic DoggyboneTM [21]) with minimal length are also developed by removing the bacterial backbone. However, eukaryotic expression vector used for the generation of DNA vaccines contains promoter at 5' end (CMV), gene of interest (viral DNA) and 3' poly (A) tail [19].

Delivery — The investigation and experimentation on DNA vaccines were initiated in early 90's. At that time, intramuscular and intradermal route of incorporation was preferred. However, the low immunogenicity is observed in clinical administration of the DNA vaccine alone. Generally, for effective functionality, DNA vaccine must cross two membranes (*viz.*, plasma and nuclear) to regulate the protein expression whereas, RNA vaccines are immediately translated after crossing the plasma or endosomal membrane. Thus, to increase the chances of successful gene delivery, uptake and immunogenicity of DNA vaccines, the multiple gene delivery techniques (viz., gene gun, jet injection and electroporation) has been developed and implemented. Out of which, electroporation based *in vivo* (preclinical and clinical) gene

delivery is well accepted and shows favorable outcomes [14,22]. Additionally, the administration of DNA vaccines is tested through multiple strategies such as lipid nanoparticles (cationic lipids and cholesterol), polymeric absorption (PEI) and adsorption of biocompatible nanoparticles (PLGA and chitosan), which increases the DNA uptake and antigenic expression [23]. Furthermore, PRR and IL-12 cytokine as molecular adjuvants are also preferably combined with the antigen to guide it directly towards the APC, to initiate the immunological response. Additionally, these vaccines are documented to use in association with protein or viral vector based vaccines, which gives synergistic boost to the immune system [24].

Mechanism — Several studies report that, both innate and as well as humoral immunity is induced through the administration of DNA vaccine. Both CD8+ (cytotoxic) and CD4+ helper T cells get activated but the exact mechanism has not been established yet. After incorporation, these vaccines are recognized by multiple components of innate immune system. Moreover, reports show that STING/TBK1/IRF3 pathways and the AIM2 inflammasome are involve and regulate the efficacy of these vaccines [24]. ID administration of these vaccines is taken up and processed by APCs (macrophages, monocytes, and dendritic cells), which binds to the naïve T cells and leads to the activation of adaptive immunity. However, subcutaneous administration of these vaccines is taken up by fibroblast and keratinocytes, which generates antigenic responses that are recognized by APCs to initiate the immunological responses. Vaccine administered via transdermal delivery is taken by Langerhans cells that further processes and expresses the antigen. Several studies reported that APCs are primarily responsible for the activation of MHC-I restricted CD8+ T cells in the DNA vaccinated individuals. However, the cross-priming and APC mediated presentation of both MHC-I & II restricted antigens are the primary mechanisms involved. Overall, extensive study of involved mechanism is still needed [25–29].

Clinical status of DNA vaccines against SARS-CoV-2 — Globally, pharmaceutical companies and research institutes are providing their efforts to develop DNA-based vaccines against SARS-CoV-2. Currently, some DNA based vaccines are in the development stages *viz.,* AG0302-COVID19 (AnGes), INO-4800 (Inovio), ZyCoV-D (Zydus Cadila), and GX-19 (Genexine) vaccines are in phase III clinical trials; COVID-eVax (Takis), GLS-5310 (GeneOne Life Science Inc), VB10.2210 (Nykode Therapeutics), VB10.2129 (Nykode Therapeutics), Covigenix VAX-001 (Entos Pharmaceuticals Inc), and AG0301-COVID19 (AnGes) vaccines are in phase II clinical trials; CORVax12 (Providence Health & Services), COVIGEN (University of Sydney), COVIDITY (Scancell), and bacTRL-Spike (Symvivo) vaccines are in

a phase-I clinical trial [30]. Among them, only ZyCoV-D (Zydus Cadila) has been approved for emergency use in India and has shown overall 66.6% efficacy. It encodes the genes specific to spike protein and IgE signal unit. The intradermal administration of 3 doses over the course of 56 days has shown robust cellular and humoral immune responses. The ongoing registered clinical trials of ZyCoV-D (Zydus Cadila) vaccines include two Phase I and II and one Phase III [31,32]. (Table 2)

Adverse effects — Interim result of 28000 individuals (≥12 years of age) vaccinated with ZyCoV-D DNA vaccine showed 66.6% efficacy in symptomatic individuals and 100% in moderate disease after a third dose [33]. *In phase I trial, 14·58% and 12.5%* individuals showed at least one solicited and one unsolicited adverse event. However, no serious complications were reported [31].

Merits and Demerits — These vaccines offer number of advantages in context to their production and development. Their development and production platform is a rapid process in comparison to other types. They are attractive, reliable and a time-saving effort, in cases like spread of viral diseases including life-threatening SARS-CoV-2 like pandemics where a huge amount of vaccine production is required for mass vaccination. They are safe, well-tolerated, highly adaptable to new pathogens like SARS-CoV-2 and are stable at room temperature [14,15].

It has a few disadvantages like low immunogenicity and difficulty in administration inside the body. However, the genomic incorporation is a bigger risk or clinical challenge observed in the DNA vaccinated individuals [34–36]. The long-term existence of DNA plasmids in the host cells after injection may leads to several problems. Pre-clinical studies states that after IM administration, plasmid was present in the system for 12 month with low immunogenicity [37]. FDA confirms its presence only at the site of injection for more than 60 days but not in other parts the body. However, the long-term existence of genetic material in nucleus of the cells may have the chances of genomic integration, mutagenesis and oncogenesis. Recently, the genomic integration upon IM injection of genetic material through electroporation has been detected in mice model. As a safety measure, FDA recommends genomic integration studies if >30K copies of plasmid DNA/μg of host DNA is present in any tissue. Furthermore, the immune system can reject/neutralize the plasmid DNA as it is originated from bacterial system. However, no such case has been reported during preclinical studies in mice, rats, rabbits and non-human primates. Previously, the key expression of antibiotic resistance marker in the immunized individuals is raised as a safety issues but now

it is not observed due to its removal/replacement of these markers in next generation vaccines. Furthermore, the other adverse effects such as immune suppression, chronic inflammation and autoimmune diseases may be generated due to the induction of higher expression of cytokines after the DNA based vaccination. As a safety measure, WHO has recommends to determine the persistence time of a cytokine expressing plasmids in the relevant animal models [35,36].

Storage — 2-8°C temperature.

2.1.2. RNA vaccines

RNA vaccines contain genetic material which encodes the viral mRNA and are capable of translating antigenic proteins in humans, resulting in the stimulation of our immune system to fight against viral diseases. mRNA acts as an intermediary biomolecule between DNA and proteins. These vaccines deliver the antigen after translation of mRNA on ribosome. It also activates $CD8^+$ T cells after vaccination *via* MHC-I & MHC-II pathways. These vaccines are simple, cheap, and can fulfill the requirement of a huge number of vaccines to mass vaccinate the human population throughout the world in this SARS-CoV-2 pandemic [38–42].

Production — These vaccines designed against specific pathogen may contain either non-replicating or self-amplifying mRNA. Non-replicating mRNA consists of antigen coding sequence with 5' and 3' UTRs. Generally, they are produced by the transcription of cDNA which is obtained from the plasmid DNA of E. Coli [39]. Transcription of cDNA into mRNA is achieved via recombinant phage (T7 or T3 or Sp6 phage) DNA-dependent RNA polymerase and NTPs [43]. In order to remove the reaction impurities and get pure forms of mRNA, liquid chromatography techniques (FPLC and HPLC) are implemented [44]. This transcribed mRNA product consists of protein-encoding open reading frame, UTRs at both 5' and 3' end and 3'poly (A) tail [45,46]. On the other hand, the self-amplifying mRNA vaccines are generally based upon the alpha viral genome where the genes which encode the structural and functional proteins are replaced by the antigen (mRNA). Here, the viral RNA polymerase plays a crucial role in the transcription and replication of mRNA. The full-length mRNA used in this type of vaccine is 9-10 kb, which is generally greater than in non-replicating mRNA vaccines. However, it contains UTRs at 5' and 3' ends, a cap and poly (A) tail [47–49]. Generally, the production of self-amplifying mRNA vaccines is very low due to the larger size of mRNA coding sequence than the non-replicating mRNA vaccines. Generally, the encoded mRNAs are supposed to contain sub-genomic promoter and ORF for the transcription of several NSPs

through the utilization of RdRP enzyme. It leads to the generation of negative strand copies which acts as template for the synthesis of two positive strands and leads to the amplification of mRNA that encodes the antigen [50,51].

Delivery — In order to achieve the protein expression, the mRNA vaccine must be delivered in cytosol. Once it crosses the plasma and endosomal membrane, it has capability to stimulate the innate immune cells. The ability of mRNA vaccines to stimulate the cellular immunity and delivery may be enhanced through the several ways. Generally, this type of vaccination is achieved through direct injection via ID and intranodal route to the APCs [52–57]. Furthermore, gene gun and electroporation methods are also preferred to enhance the administration of mRNA vaccine to cytosol [58–60]. However, the IM administration of LNP formulated mRNA is much effective to induce both innate as well as humoral immunity than the naked mRNA [61]. Recently, novel strategies like use of mRNA with other adjuvant molecules have been developed and found to work efficiently. Lipid or polymer based nanoparticles of mRNA vaccine are studied and documented to improve the efficacy, cellular uptake and delivery to the cytosol [62]. For the cell-based delivery of mRNA various polymers (Lipofectamine) and cationic lipids are preferred, while their use is not preferred for *in vivo* delivery due to cytotoxicity and less transfectability. For *in vivo* delivery of mRNA and si-RNAs, lipid-based nanoparticles are effective and well tolerated. Generally, LNP based formulations consists of ionizable amino lipids, phospholipids, cholesterol and PEG [61,63,64].

Additionally, the knowledge of route of administration along with the formulation is also important to improve the efficacy and safety of mRNA vaccines. IV administration of lipid-=based nano-formulation targets the liver while the administration of similar formulation through the ID and IM route promotes the durable expression of antigen of interest at the site. Thus, the selection of right route of administration matters for the generation of desired outcome [61,65–67]. However, IM route is most practiced, easy to deliver and preferred route in COVID19 pandemic for the mRNA-based vaccines. IM injection of mRNA based vaccines is studied for the strong induction of immune cells at the site of injection [61,65,68].

Mechanism — Exogenous mRNA in the form of vaccines is recognized by several PRRs present in endosome and cytosol. mRNA vaccines mediated activation of PRRs leads to the generation cytokines (IL12, TNF) and chemokine's at the site of injection acts as an innate immune factors for the induction of adaptive immunity [69,70]. ID administration of mRNA vaccines is documented to enhance the expression of multiple chemokine's (CXCL9, CXCL10,

and CXCL11), which promotes the recruitment of dendritic cells and macrophages to the site of administration [71]. Furthermore, the injection of protamine based non-replicating mRNA vaccines is reported to be taken by both leukocytic as well as non-leukocytic cells and later on presented by APCs [72]. Then, these mRNAs are transported by migratory DCs to lymph nodes (dLNs) which further leads to the activation of adaptive immune responses after the proliferation of T cells and innate immune cells. Notably, the activation of immune cells is primarily noticed to at the site of administration and lymphoid system in the pre-clinical mice model [68]. Moreover, the IM injection of self-amplifying mRNA vaccine is documented to restrict the CD8+ T cell priming and helps in the antigen transfer from myocytes to APCs in mice. Recently, the higher expression of non-leukocytic cells, neutrophils and professional APCs were also observed at the site of IM injection of lipid nanoparticles based mRNA vaccines [61]. Similarly, the recruitment of various immune cells such as neutrophils, monocytes and DCs to the site of injection were also observed in non-human primates upon lipid nanoparticle based non-replicating mRNA vaccination [73]. After internalization, translation of mRNA is followed by the upregulation of CD80 and CD86 co-stimulatory receptor molecules. Simultaneously, the upregulated expression of type-1 ISGs was also observed. Therefore, the transiently expressed innate immune cells lead to the T-cell priming to cause the activation of B-cells to produce antigen specific antibodies [61,74].

Clinical status of RNA vaccines against SARS-CoV-2 — In the last two decades, RNA vaccines were documented for SARS or MERS-CoV. But, in this SARS-CoV-2 pandemic, a number of RNA vaccines are under study and some of them are in developmental stages: LNP-nCOV saRNA-02 Vaccine (MRC/UVRI and LSHTM Uganda Research Unit), HDT-301 (SENAI CIMATEC), PTX-COVID19-B (Providence Therapeutics Holdings Inc), mRNA-1283 (Moderna), mRNACOVID-19 Vaccine (Stemirna Therapeutics Co Ltd), mRNA-1273.351 (Moderna), and CoV2 SAM (LNP) (GlaxoSmithKline) are in phase-I clinical trials; Inc EXG-5003 (Elixirgen Therapeutics), TAK-919 (Takeda), DS-5670a (Daiichi Sankyo Co Ltd), ARCT-165 (Arcturus Therapeutics Inc), BNT162b3 (Pfizer/BioNTech), BNT162c2 (Pfizer/BioNTech), HGCO19 (Gennova Biopharmaceuticals Limited), LUNAR-COV19/ARCT-021 (Arcturus Therapeutics Inc), BNT162a1 (Pfizer/BioNTech), and ChulaCov19 (Chulalongkorn University) are in phase-II clinical trials; BNT162b2 (Pfizer/BioNTech), BNT162b1 (Pfizer/BioNTech), mRNA-1273 (Moderna), mRNA-1273.617.2 (Moderna), mRNA-1273.211 (Moderna), ARCT-154 (Arcturus Therapeutics Inc), mRNA (Walvax), and BNT162b2s01 (Pfizer/BioNTech) are in phase-III trial (see Table 2) [30].

Among them, mRNA-1273 (Moderna) carries the nucleoside-modified mRNA of spike protein has been approved in 79 countries. Two doses of the vaccine administered intramuscularly over 28 days show 94.1% overall efficacy and enhanced CD4+ T cell responses against Th1 cytokine. Registered clinical trials: phase-I(6), Phase-II(18) and Phase-III(10) [75,76]. Dual dose of mRNA-1273 vaccine showed 98.4% effectiveness in alpha, 95.5% in gamma and 86.7% in delta variants [77]. A Qatar based study showed 100% effectiveness against alpha and 96.4% effectiveness against beta variants [78]. Additionally, mRNA-1273 vaccine also showed 93% efficacy against omicron [79]. Another vaccine containing the same targeted sequence, TAK-919 (Takeda) has been approved by Japan for emergency use. Two intramuscular doses resulted in elevated neutralizing antibody titters and also induced CD4+ T-cells. As the vaccine is still under trial, the efficacy percentage has not been established. Currently, registered for phase-I and phase-II clinical trials [80,81]. Most widely accepted RNA vaccine, approved in 115 countries is BNT162b2 (Pfizer/BioNTech) which showed 95% overall efficacy and enhanced level of SARS-CoV-2 neutralizing antibody and prominent antigen-targeted CD8+ and Th1-specific CD4+ T-cell responses when intramuscularly administered. Registered clinical trials are: Phase-I(9), Phase-II(26) and Phase-III(11) [82,83]. BNT162b2 had 88% efficacy against delta variant in comparison to 93.7% against alpha variant [84]**.** Another study reported that BNT162b2 vaccine had 89%, 100% 63% and 92% efficacy against alpha, beta, gamma and delta variants respectively [85]. Moreover, another result showed it had 70% efficacy against Omicron variant [86]**.** In addition, vaccines LNP-nCoVsaRNA (Imperial), CVnCoV (Curevac), and MRT5500 (Sanofi Pasteur) are not passed in trials [30]. Development of RNA-based vaccines is still in progress; hopefully it will end up with safer and effective outcomes.

Clinical trials are often affected by the confinements and nature of investigation which may influence expected results in real life scenario. Determination of vaccine efficacy and associated adverse effects in post-public vaccination are being estimated worldwide through real-life interim analysis. In Israel, BNT162b2 mRNA vaccine efficacy was estimated in 596618 individuals, vaccinated between 20th December 2020 and 1st February 2021. 92% overall vaccine efficacy, 94% against symptomatic group, 87% against hospitalization and 92% against severe disease was estimated [87]. Meta-analysis of 19 large scale observational studies showed 95% overall efficacy of BNT162b2 vaccine [88]. In a study where, 352878 individuals who received 2 doses of mRNA-1273 vaccine were matched with similar number of unvaccinated candidates, 87·4% effectiveness against infection, 95·8% against hospitalization

and 97.9% against death was found. Moreover VOC specific effectiveness was found to be 100% against alpha variant and 96.4% against beta variant [89]. A study reported meta-analysis of 23 real-life study of BNT162b2 mRNA vaccine showed 91.2% efficacy against infection, 97.6% against hospitalization and 98.1% against associated death. Similarly, analysis of five articles related to mRNA-1273 (Moderna) mass-vaccination, showed 98.1% effectiveness [90]. Commonly reported side effects of RNA vaccine included tenderness, weakness, fever, palpitations, headache, joint/muscle pain, nausea, anorexia, insomnia, local swelling, tingling/itching, diarrhoea and nasal congestion [91,92].

Immune response persistence, rapid waning and booster vaccination — A study on approx. six lakhs dual dose vaccinated individuals with RNA based vaccines [(*viz.,* BNT162b2 (33%), ChAdOx1 nCoV-19 (65.3%) and mRNA-1273 (1.7%)] is performed to detect the immunity persistence, waning and their risk factors. Subsequently, in this study SARS-CoV-2 diagnostic tests were performed upto 6 months in certain time gaps immediately after the vaccination with dual doses of respective vaccines. After six months, it is found that 10% of vaccinated individuals were tested positive. Effectiveness of all these vaccines was noticed to waned after five months. Effectiveness of BNT162b2 was 82.1%, ChAdOx1 nCoV-19 is 75.7%, and mRNA-1273 is 84.3% studied after five months. In this study, their effectiveness was also studied in various age groups and found to be much waned in older aged persons (age $\geq$ 55) and in persons with other co-morbidities. It is also found that effectiveness of BNT162b2 primary dose is boosted to more than 92.5% by receiving their booster dose after 3 months. Similarly, the effectiveness of ChAdOx1 nCoV-19 was boosted to more than 88.8% after receiving the booster dose in 3 months gap. Additionally, adverse effect was observed in 10.1% participants. Muscle tenderness was observed as most common adverse effect in 59.2% participants. However, it was also observed that the heterologous booster doses results in much systemic adverse effects than the homologous schedules of vaccines. Similarly, the effectiveness and safety of BNT162b2 was waned to 53.4% and 16.5% after one and three months of post-vaccination respectively. This study was performed during the period of omicron variant and the immunity persistence was waned to much larger extent. Thus, it can be concluded that the RNA based vaccines persists immunity for more than six months in young individuals and the booster doses at regular interval of time are capable of boosting the safety, effectiveness and immunity against SARS-CoV-2 [93,94].

Hybrid immunity — Immunity against SARS-CoV-2 can be induced by naturally (infection mediated) and passively (vaccine mediated). However, the term ‘hybrid immunity’ used for

immunity acquired in individuals who had received one or more doses of vaccines and had experienced SARS-CoV-2 infections at-least once before and after immunization. Recently, hybrid immunity was studied to provide more protection in comparison to both natural and passive immunity. It also observed during the period of delta variant that the superior immune protection of people with hybrid immunity in comparison to un-infected persons vaccinated with dual doses or infected persons not vaccinated at all. Additionally, the reduction in the efficacy of hybrid immunity was observed during the period of omicron variants but its magnitude and duration is not well studied yet. Thus, much validation and precise measurement of hybrid immunity is needed to be done [95].

Adverse effects — Occurrence of Pericarditis/Myocarditis events in adolescent/young adult males have been reported as unusual complication of COVID-19 associated mRNA vaccines. According to CDC, reporting rate of myocarditis cases/million mRNA vaccine second dose was found to be 62.8 and 50.5 among 12−17 and 18−24 year's old males, respectively [96]. After 2 to 3 days of second dose, vaccine induced myocarditis patients had symptomatic chest pain in association with elevated ST segment and levels of cardiac troponin [97]. In Israel, among 5.1 million individuals vaccinated with dual dose of BNT162b2 mRNA vaccine, 136 recipients showed definitive myocarditis symptoms post-vaccination and incidence ratio was found to be 5.34 in males aged 16-19 years [98]. Moreover, 23 male US military personnel (20-51 years old) were diagnosed with myocarditis symptomatic chest pain within four days of vaccination with BNT162b2 or mRNA-1273 vaccine [99]. This risk was further estimated to increase by 18.28 folds in vaccinated individuals previously infected with SARS-CoV-2 [100].

Arrhythmia is another rare adverse event associated with COVID19 vaccination. This could be a result of impaired vasoconstriction due the autoimmunity induced damage of cardiovascular adrenergic receptors [101]. Among BNT162b2 vaccinated candidates, three individuals who had a history of SARS-CoV-2 infection developed tachycardia [102]. Similar postural orthostatic tachycardia event was observed in a patient, 6 days after first dose of BNT162b2 [103].

Myocardial Infarction has also been reported as a rare complication of AstraZeneca, Pfizer and Sinovac vaccines administration and the occurrence post vaccination varied between 15 minutes to 2 days [104–107]. Risk ratio was determined to be 4.47 in Pfizer vaccine recipients with COVID19 infection history and 1.07 without [100].

Stage III hypertension was presented minutes after vaccination with Pfizer/BioNTech vaccine in eight patients and Moderna vaccine in one patient [108]. In another group, among 113 individuals, 6 average elevation of systolic/diastolic blood pressure by ≥ 10 mmHg [109]. Rare case Takotsubo cardiomyopathy has also been reported after vaccination with Moderna and AstraZeneca vaccines [110,111].

Merits and Demerits — The non-replicating mRNA vaccines are much preferred over the self-amplifying mRNA vaccines due to their small and simple construct which promotes the generation of immune specific responses. Furthermore, the lD and intranodal administration of naked mRNA is studied to induce the immune responses but they are highly un-protective due to the presence of ribonucleases in the extracellular matix. Also, they are highly unstable in the biological environment because of their negative charge and 'water loving' nature. However, the lipid nanoparticle based complexation of mRNA is preferred, to overcome this stability issue [52–55]. Generally, the mRNA vaccine-based activation of pattern recognition receptors (PPRs) leads to the activation of innate immunity and followed by activation of acquired immunity. Along with this, activated PPRs are also reported to activate the type-1 interferons, followed by the phosphorylation of eiF2a, which results in reduction and inhibition of protein synthesis. Thus, it concludes that mRNA based vaccines works like a dual-edged sword [112]. Furthermore, self-amplifying mRNA-based vaccines induced rapid inflammatory immune responses through the activation of several ISGs. It is suggesting that the potency of these vaccines can be improved by reducing the early type-1 interferon responses. Furthermore, the basic production platform of these vaccines is quiet similar to the DNA based vaccines. However, the production of these vaccines does not require microbial system for amplification. Its production is quiet simple and easy to handle than the DNA vaccines. And no chances of genomic integration are there in RNA vaccines because it does not interact with host DNA. Furthermore, the chances of anti-vector immune responses generation are very much low than in the viral vector-based vaccines. So, these vaccines can be administered number of times because of less or no chances of development of pre-existing immunity. These vaccines do not require any special device such as gene gun and electroporation, instead of these methods they can be injected through the multiple routes by normal injection syringes. Thus, the utilization of these vaccines is much preferred than other vaccine strategies in pandemic situation such SARS-CoV-2 [40,113–116].

These vaccines are also safe, well-tolerated, and flexible to new pathogens and their efficacy is also enhanced by lipid, protamine, and polymer-based nanoparticles [12,117].

Furthermore, RNA vaccines do not need host cell genome integration. This is the major advantage of RNA vaccines over the DNA vaccines. The chances of genomic mutations are lower in RNA vaccinated individuals than the DNA vaccinated individuals. RNA vaccines can be injected into the host via intravenous route, while DNA-based vaccines need electroporation or gene gun for their administration. However, these vaccines also have disadvantages like possible degradation of the mRNA and interferon's mediated antiviral immune response resulting in suppression of RNA vaccines efficacy [118]. Furthermore, activation of interferon signaling is documented for their association with inflammation and autoimmunity [38]. However, no report is documented for the RNA vaccine-based induction of autoimmune diseases. Hence, further study is needed to be done for the verification of any adverse effects caused by RNA vaccines.

Storage — mRNA molecules are quite unstable than the DNA molecules. So, long term storage of mRNA vaccines are done between −70 °C and −20 °C while the short term storage up to six months is done between 2 and 8 °C [38].

2.1.3. Subunit vaccines (Protein and Virus like particle)

Administration of whole pathogen is not an essential prerequisite of vaccine-based stimulation of immune system. Purified antigenic fragment of virus particle can act as an adequate inducer. Antigenic fragment can be a protein, polysaccharide or even virus-like particle. Moreover, combination of antigens can be used as conjugated vaccine. Major classes of subunit vaccines are discussed below:

2.1.3.1. Protein-based subunit vaccines

Protein subunit vaccines are developed by using the protein components of the viral particles like spike proteins of the virus. They are also safer and well-tolerated, although it has disadvantages too. These vaccines are documented to have low immunogenicity and require adjuvant/conjugates to enhance their immunogenicity [119–125]. Recently, one group has reported that disulfide-associated dimeric variant of MERS-RBD has improved the immunological activity of protein-based vaccines and consecutively this protocol was explored for SARS-CoV-2, resulting in 10-100 folds increment in neutralizing antibody titers [126]. Hopefully, this

platform will help in increasing the immunogenicity of protein subunit vaccines globally in this pandemic situation and future.

Previously, viral S protein subunit vaccines were well known for SARS-CoV as well as for MERS-CoV. S protein segment of MERS-CoV is documented to contain non-neutralizing antibodies, which may hinder the generation of neutralizing antibodies as well as the immunogenic response against MERS-CoV. Therefore, it is mandatory to search novel neutralizing epitopes for the development of protein subunit-based vaccines. Spike proteins expressed on SARS-CoV/MERS-CoV viral surfaces are a type-I trimeric membrane protein that binds to DPP4 on the target cells. At present, neutralizing epitopes and operational channels of monoclonal antibodies were studied at molecular level by structural and functional studies. It includes 2E6, 4C2, m336, MCA1, 7D10, D12, MERS-27, CDC-C2, JC57-14, MERS-GD27, and MERS-4. These antibodies target the RBD sub-domain of SARS-CoV/MERS-CoV and overlap with the binding surface of DPP4 [127–132]. These documented studies are crucial keys for the development of protein subunit vaccines against SARS-CoV-2.

Production — The antigenic protein part of pathogen can be generated either by culturing large quantity of pathogens or synthesis of recombinant protein. Protein subunit vaccines are produced in live microbial systems like yeast. The antigenic gene is inserted into the yeast cells and grown in large fermentation bioreactors to produce recombinant protein subunits. After purification, the subunits are combined with preservatives for maintenance of self-life and adjuvant like alum. The risk associated with whole pathogen-based vaccines is omitted by this system, however, sometimes booster doses and adjuvant become a requirement for potent immunological response. Protein based subunit vaccines have been previously designed against acellular Pertussis, Hepatitis B and Papilloma virus. Currently, 9 Protein based subunit vaccines against SARS-COV2 have been approved [133–136].

Mechanism — Protein subunit vaccines utilize the antigenic protein parts of pathogen to induce germinal center that results in the production of highly specific antibodies. Due to the limited size, major stimulators of immune systems, pathogen-associated molecular patterns (PAMPs) are absent in the subunits which makes the use of necessary adjuvants to activate APCs for internalization of the antigen and present it to cells of adaptive immunity [133–135].

Clinical status of protein subunit vaccines against SARS-CoV-2 — Till now, the clinically approved protein subunit-based vaccines are available against *Pertussis, Influenza, Streptococcus pneumoniae, Haemophilus influenza type b* [137]. SARS-CoV-2 protein subunit-

based vaccines are also being developed, among which some are in the developmental stages. Some of the SARS-CoV2 protein subunit vaccines are PIKA COVID-19 (Yisheng Biopharma), CoV2-OGEN1 (VaxForm), PepGNP-SARSCoV2 (Emergex Vaccines Holding Ltd), SpFN COVID-19 (US Army Medical Research and Development Command), CoVepiT (OSE Immunotherapeutics), IN-B009 (HK inno. N Corporation), NBP2001 (SK Bioscience Co Ltd), and SARS-CoV-2 Vax 1 (Baiya Phytopharm Co Ltd Baiya) in phase-I clinical trials; AdimrSC-2f (Adimmune Corporation), EuCorVac-19 (EuBiologics Co Ltd), AKS-452X (University Medical Center Groningen), 202-CoV (Shanghai Zerun Biotechnology, Walvax Biotechnology), QazCoVac-P (Research Institute for Biological Safety Problems), SARS-CoV-2 Protein Subunit Recombinant Vaccine (PT Bio Farma); SII Bivalent (Novavax), SII B.1.617.2 (Novavax), SII B.1.351 (Novavax), KBP-201 (Kentucky Bioprocessing), ICC (Novavax), Soberana 01 (Instituto Finlay de Vacunas Cuba), IVX-411 (Icosavax), HIPRA (Laboratorios Hipra SA), SCB-2020S (Clover), TAK-019 (Takeda), COVAC-2 (University of Saskatchewan), COVAC-1 (University of Saskatchewan), Recombinant RBD Protein Vaccine (Bagheiat-allah University of Medical Sciences), CIGB-669 (Center for Genetic Engineering and Biotechnology (CIGB)), BECOV2D (Biological E Limited), BECOV2C (Biological E Limited), BECOV2B (Biological E Limited), and BECOV2A (Biological E Limited) are in phase-II clinical trials; Recombinant Protein vaccine (Sanofi/GSK), SCB-2019 (Clover), UB-612 (COVAXX), FINLAY-FR-2 (Instituto Finlay de Vacunas Cuba), NVX-CoV2373 (Novavax), Nanocovax (Nanogen), SP/GSK subunit D614 (Sanofi/GSK), V-01 (Livzon Mabpharm Inc), ZF2001(Anhui Zhifei Longcom), Recombinant SARS-CoV-2 (CHO Cell) (National Vaccine and Serum Institute), ReCOV (Jiangsu Rec-Biotechnology Co Ltd), Razi Cov Pars (Razi Vaccine and Serum Research Institute), Soberana 02 (Instituto Finlay de Vacunas Cuba), Soberana Plus (Instituto Finlay de Vacunas Cuba), SP/GSK subunit B.1.351 vaccine (Sanofi/GSK), COVOVAX (Novavax formulation) (Serum Institute of India), MVC-COV1901 (Medigen), S-268019 (Shionogi), EpiVacCorona (FBRI), SCTV01C (Sinocelltech), CIGB-66 (Center for Genetic Engineering and Biotechnology (CIGB)), GBP510 (SK Bioscience Co Ltd), AKS-452 (University Medical Center Groningen), COVAX-19 (Vaxine/CinnaGen Co.), and Recombinant (Sf9 cell) (West China Hospital) are in clinical trial phase-III. Out of which vaccines *Viz.*, EpiVacCorona (FBRI), ZF2001(Anhui Zhifei Longcom), CIGB-66 (Center for Genetic Engineering and Biotechnology (CIGB)), Soberana Plus (Instituto Finlay de Vacunas Cuba), MVC-COV1901 (Medigen), Razi Cov Pars (Razi Vaccine and Serum Research Institute), COVOVAX (Novavax formulation) (Serum Institute of India), COVAX-19 (Vaxine/CinnaGen Co.), and Soberana 02 (Instituto Finlay de Vacunas

Cuba) are approved; and Sclamp (Queensland) vaccines are removed from clinical trials [30] (Fig. 2).

EpiVacCorona developed by FBRI has been approved for emergency use by Russian Federation and Turkmenistan. This Spike protein-based vaccine is intramuscularly administered twice over 28 days and showed Seroconversion ≥ 1:20 in 100% vaccinated individuals. Registered clinical trials are: Phase-I & Phase-II(1) and Phase-III(1) [138,139]. ZF2001 (Anhui Zhifei Longcom) containing the RBD peptide is approved in China, Indonesia, and Uzbekistan. Two/three doses over 4-week intervals results in 82% (overall), 93% (Alpha) and 78% (Delta) efficacies. Notable humoral responses (Th1 and Th2) and 2-fold increase in GMTs were observed as compared to convalescent serum. Ongoing clinical trials are in 5 countries: Phase-I(4), Phase-II(3) and Phase-III(4) [140,141]. CIGB-66 (Genetic Engineering and Biotechnology) is a RBD protein-based dual-dose vaccine, approved in 4 countries [Registered : Phase-I & Phase-II(2) and Phase III(1)] and has shown 92.28% overall efficacy [142,143]. Soberana 02 (dual dose) and Soberana Plus (single dose) developed by Instituto Finlay de Vacunas Cuba are based on RBD and dimeric-RBD protein respectively. Soberana Plus could be use a booster dose of Soberana 02 vaccinated people showed 62% overall efficacy with ≥ 4-fold seroconversion while 91.2% after Soberana Plus booster. Soberana Plus increases 60.9% to 89.2% ACE2 interaction; 94.5 to 340 mVNT50 GMT and 24.2% to 65.6% viral neutralization. Registered clinical trials- Soberana 02: one in each Phase-I, Phase-II and Phase-III(1) [144,145]; Soberana Plus Phase-I(2), Phase-II(2) and Phase III(1) [145,146]. MVC-COV1901 (Medigen) vaccine targeting the S2 subunit of spike protein which is approved by Taiwan for emergency use. It increases GMT from 163.2 to 662.3 and seroconversion rate to 99.8%. Phase-I(1), Phase-II(6) and Phase-III(1) clinical trials are currently under way [147,148]. Iran recently approved Razi Cov Pars, a spike protein-specific vaccine developed by Razi Vaccine and Serum Research Institute. Two intramuscular doses are followed by a third intranasal dose on day 51. As the clinical trial of the vaccines is still in progress, enough data on efficacy and immunogenicity is not available [149,150]. COVOVAX (Phase-II and Phase-III trials) showed 86% efficacy against UK, 60% against South Africa, as well as 89.3% against B.1.1.7, and 49.4% against B.1.351 variants. It targets the spike protein and resulted in 4-fold higher GMT level in symptomatic patients as compared to convalescent plasma. It also induced CD4+ T-cells and lead to the over expression of IFN-γ, IL-2, and TNF-α. Currently, it is approved for emergency use in India and Philippines [151,152]. Based on the increase in neutralizing antibodies and T cell responses, another spike protein-specific vaccine called COVAX-19 or SpikoGen developed

by Vaxine/CinnaGen Co. and approved for emergency use in Iran. Presently, clinical trials are progressed as Phase-I(1) in Australia; Phase-II(2) and Phase-III(1) in Iran (see Table 2) [153,154].

Adverse effects — NVX-CoV2373 a protein subunit vaccine in phase I trial creates common side effects like soreness, pain at injection site, headache, fatigue and muscle pain [155]. In MVC-COV1901 vaccinated group 71·2% individuals had pain at injection site, 36% had malaise/fatigue and 0.7% had fever [148]. In phase I, 40% out of 40 enrollees and in phase IIa 32% out of 100 enrollees reported at least one adverse effect within 28 days of SOBERANA 02 administration. Moreover, in phase IIa one recipient acquired serious, grade 3 erythema and induration [145].

2.1.3.2. Vaccines containing virus-like particles

Self-assembled VLP or virus-like particles are structural proteins of a virus that imitates the orientation of parent virus, and it also lacks a viral genome. VLP based vaccines use the epitopes in conformation which is similar to the parent virus, and that results in superior immune responses. Like whole virus vaccines, VLP based vaccines do not incorporate live or inactivated viruses that make them a safer candidate. VLP based vaccines mediate the induction of high antibody expression due to cross-linking with B-cell receptors. These vaccines have disadvantages like other types of vaccines. They have low immunogenicity and secondly its manufacturing process is too much typical [156–158].

Production — Viral vaccines are based on artificial non-viral molecules that closely mimic viruses, but do not cause infection due to the lack of genetic material. Generally, the vaccines are made up of structural parts which assembles to form empty virus. The production method mainly comprises of different expression platforms like bacteria (*E. coli*), yeast, insect cells, mammalian cells and plants (*viz.,* tobacco mosaic virus, TMV) and the choice of these systems depends on required post translation modifications and protein folding mechanism. VLP based vaccines have been previously designed against Hepatitis B, Malaria, Human papilloma viruses, Influenza virus A and Human Immunodeficiency Virus (HIV) and currently clinical trial of vaccine against SARS-COV2 is in process [159–164].

Mechanism — VLP cannot replicate but presents dense and repetitive conformation of structural protein epitopes which can stimulate potent cellular and humoral responses without adjuvant. Moreover, VLP size ranges from 20 to 200 nm which can easily transferred to

draining lymph nodes. After the transfer it enters subcapsular sinus where it is caught by macrophages [165].

Delivery — these vaccines are usually administered intramuscularly to avoid local adverse effects [166].

Clinical status of VLP vaccines against SARS-CoV-2 — Till now, the clinically accepted VLP vaccines are against *human papillomavirus and hepatitis-B virus* [156–158]. However, in this COVID-19 scenario, VLP based vaccines are also in the developmental stages to combat the SARS-CoV-2 virus are VBI-2902a (VBI Vaccines Inc), RBD SARS-CoV-2 HBsAg VLP (SpyBiotech), ABNCoV2 (Radboud University), SARS-CoV-2 VLP Vaccine Alpha Variant (The Scientific and Technological Research Council of Turkey) and SARS-CoV-2 VLP Vaccine (The Scientific and Technological Research Council of Turkey) are in phase-II; LYB001 (Yantai Patronus Biotech Co Ltd) is in phase-I and Plant-based VLP (Medicago) vaccine is currently in phase-III clinical trial [30]. These VLP based vaccine candidates are not approved yet.

Merits and demerits — Use of targeted antigen induces sensitive and specific immunological response. The absence of viral genome makes the vaccines a safe choice for vaccination of elderly, children, pregnant/nursing women and patients with underlying diseases or immune system disorder. Moreover, production technology is well established and time efficient. However, isolated proteins slowly getting partial denaturation that may stimulate non-specific antibody production. The lack of PAMPs makes these vaccines less immunogenic and requires aid of adjuvant and additional booster doses to achieve required immune responses. Pre-development identification of liable antigen is a necessity and this process may take extensive time [156,167,168].

Storage — Subunit vaccines are temperature sensitive hence require refrigeration (2-8°C) for storage [168].

2.1.4. Viral vector vaccines (replicating and non-replicating)

This vaccine platform is a highly versatile and can deliver one or more antigens, forming a multi-vaccine system which has several advantages over other vaccines. Additionally, it can include both replicating and non-replicating viral vectors for vaccine development. Since 1980s, a wide variety of viral vectors has been engineered to encode the antigen in humans.

After successful delivery and antigen expression, the host system automatically induces the immunological responses against the antigen [169].

Viral vectors — Some of the common viruses used for vector-based vaccine development viz., adenovirus, measles virus, and vesicular stomatitis virus (VSV). A brief detail of such system is given below: -

Adenovirus (Ad) — These vaccines are commonly reported for the application of Ad vectors in wide array of preclinical and clinical research. These vectors can give stable expression up to 8Kb of antigenic gene sequence. It operates in two distinct modes *viz.*, as replication competent and defective vector. The early transcript 1A and 1B (E1A, E1B) gene is usually replaced by target gene for encoding the antigen and disables the replication ability. Moreover, E3 gene is deleted to protect the Ad infected host cells from immune system. Additionally, deletion of E4 gene from Ad vector prevents any leaky expression of gene of interest [170–172].

Measles virus (MV) — It is a negative-sense ssRNA (~16 kb) based non-segmented enveloped virus that used to develop these vaccines by sequential passaging of MV virus in various cell lines. This allows integration of various mutations in the virus, generating a live attenuated viral vector, which cannot replicate in human and is non-pathogenic. MV vectors can allow insertion of gene sequence up to 6 kb abiding by the *rule of six*. This vector system allows development of multivalent vaccines which can be generated in Vero/MRC-5 cell lines or in chick embryonic fibroblasts. These vectors are usually generated in mammalian cell lines like HEK293 [171,173–175].

Vesicular Stomatitis Virus (VSV) — A negative sense ssRNA virus of ~11 kb genome size has been widely utilized as an attenuated vector for translation of 4-5 kb gene of interest. The viral attenuation can be attained by mutating the viral matrix protein, reorganizing the viral protein order, integrating non-viral protein and deleting viral glycoproteins which measures infection potential. Commonly, the glycoprotein gene is replaced by transgene, subsequently changing viral tissue tropism. VSV can be generated in insect and mammalian cell lines [176–179].

Mechanism — Based on the serotype employed, adenoviral vectors can induce varied level of T cell responses and antibody production. Ad5, a replication deficient vector can induce remarkably efficient antibody production and CD8+ T cell responses. Nonetheless, humans having pre-existing immunity against this virus may inactivate the vector and restrain expression of transgene. To overcome this limitation, non-human AV vectors like ChAd63, chimpanzee virus derived vector and unusual serotypes like Ad26 or Ad35 having low human

prevalence can be used. As compared to Ad5, use of such serotypes can enhance memory and multi-functionality of CD8+ T cells [180–184].

Recombinant MV can potently stimulate the levels of cellular and humoral immune responses against antigenic transgene. MV can directly deliver transgene into APCs by infecting dendritic cells and macrophages. Moreover, they activate CD4+ T cell-mediated responses. In some countries, children vaccination programs used MV based vaccines to induce immunity against MV. However, clinical studies of MV based vaccines for CHIKV have shown that such pre-acquired immunity did not affect vaccine efficacy [173,181,185,186]. VSVs have reported to induce both CD4+ and CD8+ T-cell responses [181].

Delivery — Clinical studies of viral vector-based vaccines can use multiple routes of delivery *viz.*, IM, intranasal, oral and ID. The type of immunological responses is dependent on the mode of vaccination, and the selection of route of administration. However, delivery of the vaccine should ideally be reliable, easy and requires no such special training in the pandemic situations of SARS-CoV-2 [187–189]. Furthermore, these vaccines do not require adjuvants for the supra-addition in their immunological responses as they itself induces strong immune responses. Recently, these vaccines in association with immune-stimulating agents were clinically tested, but no significant increment in immune responses was noted. However, adjuvant based changes in the immunological compartments is documented, but still the mechanism behind this is unknown [190,191].

Clinical status of replicating viral vector vaccines against SARS-CoV-2 — These vaccines contain SARS-CoV-2 gene(s) in a replicating viral vector which produces strong immunity and mimicry of natural infection. Typical manufacturing processes and chances of genomic integration are two major disadvantages of these vaccines [192,193]. DNA sequence-loaded viral vector vaccine may encode one or more antigens which lead to the activation of immune system. These vaccines induce a strong immune response through the activation of both T and B cells [194,195].

Some of these vaccines are Covid-19/aAPC (Shenzhen Geno-Immune Medical Institute), MV-014-212 (Meissa Vaccines Inc), and DelNS1-nCoV-RBD LAIV (The University of Hong Kong) in phase-I clinical trials; vaccines AdCLD-CoV19 (Cellid Co), DelNS1-2019-nCoV-RBD-OPT (Wantai), and COH04S1 (City of Hope Medical Center) are in phase-II; vaccines IIBR-100 (Israel Institute for Biological Research (IIBR)) and DelNS1-2019-nCoV-RBD-OPT (Wantai) are in phase-III clinical trials. None of them are approved yet

by any country. Three replicating viral vector-based vaccines V591 (Merck Sharp & Dohme Corp), COVID-19-101 (Institut Pasteur), and V590 (Merck Sharp & Dohme Corp) are no longer in progress due to their inefficiency [30]. Still, the development of such vaccines is in progress, hopefully may demonstrate to be an effective result against SARS-CoV-2.

Clinical status of non-replicating viral vector vaccines against SARS-CoV-2 — These vaccines contain SARS-CoV-2 gene(s) in a non-replicating viral vector. Chances of genomic integration are also high with the use of these vaccines. They are potential vaccine type to produce strong immunogenic responses [192,196,197]. Such SARS-CoV-2 vaccines are MVA-SARS-2-S (Universitatsklinikum Hamburg-Eppendorf), VXA-CoV2-1 (Vaxart), SC-Ad6-1 (Tetherex Pharmaceuticals Corporation), Ad5-nCoV (AMMS), ChAdV68-S (NIAID), ChAd-triCoV/Mac (McMaster University), Ad5-triCoV/Mac (McMaster University), COVID-19-EDV (EnGeneIC), CVXGA1 (CyanVac LLC), AdCLD-CoV19-1 (Cellid Co), BBV154 (Bharat Biotech) and SAM-LNP-S (NIAID) in phase-I clinical trials; hAd5-Covid-19 (Immunity Bio Inc), MVA-SARS-2-ST (Universitätsklinikum Hamburg-Eppendorf), VXA-CoV2-1.1-S (Vaxart), COVIVAC (Institute of Vaccines and Medical Biologicals), BCD-250 (Biocad), NDV-HXP-S (Mahidol University) and LV-SMENP (Shenzhen Geno-Immune Medical Institute) in phase-II clinical trials and vaccines GRAd-COV2 (ReiThera), Covishield (Serum Institute of India), Ad26.COV2.S (Janssen (Johnson & Johnson)), AZD1222 (Oxford/AstraZeneca), Sputnik V (Gamaleya), Ad5-nCoV (CanSino), Ad5-nCoV-IH (CanSino), AZD2816 (Oxford/AstraZeneca) and Sputnik Light (Gamaleya) are in phase-III clinical trials. Six non-replicating viral vector-based vaccines Ad5-nCoV (CanSino), Sputnik V (Gamaleya), Ad26.COV2.S (Janssen (Johnson & Johnson)), AZD1222 (Oxford/AstraZeneca), Sputnik Light (Gamaleya), are approved for their use and AdCOVID (Altimmune Inc) vaccine is presently out of trials due to its less effectiveness [30]. Still, some of these vaccines are in the developmental stages.

All the approved vaccines are designed against SARS-CoV-2 spike protein among which Ad5-nCoV, showed the presence of anti-SARS-CoV-2 spike receptor IgG and neutralizing antibodies after 28 days of vaccination. Owing to its 65.7% efficacy against moderate and 91% against severe symptoms, it was approved for use in 10 countries. Presently, 11 trials [Phase-I(4), Phase-II(5), and Phase-III(2)] are under process in 6 countries [198,199]. The Russian vaccines Sputnik V (dual dose) and Sputnik Light (single dose), developed by Gamaleya are popular vaccines, widely being used in 74 and 21 countries respectively. Additionally, Sputnik Light could also be used as a third booster dose of Sputnik V. Robust

cellular and humoral immune responses and 91.6% overall efficacy was observed in Sputnik V vaccinated people whereas quicker humoral response, 100% binding, and 81.7% neutralizing antibody responses and 13.1-fold increments in GMTs of seropositive participants with 79% efficacy against infection, 88% against hospitalization and 85% against death was observed in the case of Sputnik Light. Ongoing registered trials of Sputnik V includes Phase-I(4), Phase-II(12) and phase-III(6) [200,201] and Sputnik Light clinical trials include Phase-I(1), phase-II(2) and Phase-III(1) [202,203]. The Janssen vaccine, Ad26.COV2.S, currently approved in 85 countries given either single or double doses (56 days apart) showed an increase of spike protein-specific antibodies and neutralizing antibodies; GMTs from 2432 to 5729 and 242 to 449, respectively. Furthermore, its overall efficacy was found to be 66.9%. Phase-I(5), Phase-II(5) and Phase-III(6) clinical trials are registered across 18 countries [204,205]. AZD1222 and Covishield are both based on Oxford/AstraZeneca formulation. AZD1222 results in an overall efficacy of 70.4% in which two standard doses had 62.1% efficacy and a standard dose followed by a low dose showed 90.0% efficacy [206]. Covishield had 61% overall efficacy and 76% against symptomatic infection [207]. AZD1222 has been approved in 127 countries and has ongoing 52 [Phase-I(9), Phase-II(31) and Phase-III(12)] clinical trials in 23 countries [208]. On the other hand, Covishield is approved in 47 countries and has been registered for Phase II and III trials [209]. Covishield showed 81% vaccine effectiveness against delta variant [189]. Dual dose of ChAdOx1 nCoV-19 vaccine leads to 74.5% efficacy against alpha and 67% against delta variant [84]. Another study reported that this vaccine had 91%, 100%, 41% and 95% efficacy against alpha, beta, gamma and delta variants respectively [85]. Multiple studies have shown that ChAdOx1 nCoV-19 vaccine is not effective against Omicron variant [210,211]. Detailed data regarding effectiveness of other viral vector based vaccines like Ad5-nCoV, Sputnik Light, Sputnik V, Ad26.COV2.S and Covishield is not yet available in context of VOCs.

Adverse effects — In a study, 20.9% out of 599 vaccinated German healthcare workers received a viral vector vaccine (AstraZeneca), reported that (i) 87.2% overall systemic side effects including fatigue, headache, chills, fever, muscle/joint pain, nausea, malaise and lymphadenopathy; (ii) 24.8% individuals developed at least one non-communicable disease like allergy, asthma, blood/ bone/ cardiac/ bowel/ hepatic/ neurologic/ ophthalmic/ otolaryngologic/ renal/ thyroid diseases, cancer, chronic hypertension, chronic obstructive pulmonary disease, dermatologic disorder, diabetes and rheumatoid arthritis; (iii) 70.4% recipient showed local side effect like pain, swelling and redness at the site of injection and (iv) 12.4% and 3% developed at least one oral and skin related side effect [212]. In phase III

clinical trial of ChAdOx1 nCoV-19, transverse myelitis was reported in two recipients [213]. Adenoviral vector-based vaccines have also been associated with development of rare Guillain-Barre syndrome (GBS). In US, 132 reports of GBS surfaced after mass vaccination with 13.2 million doses of Ad26.COV2.S vaccine. The rate was estimated to be 9.8 cases/million doses between 45 to 62 years of age and the median onset time was stated to be 13 days post vaccination. Among these 35% were severe and death of one patient was reported. Moreover, bilateral facial weakness was also observed in few cases [96,214].

Vaccine induced generation of spike protein can interact with ACE2 receptor leading to cardiovascular complications like inflammation, aggregation of platelets and thrombosis. Thrombotic thrombocytopenia has been reported as an adverse effect of adenoviral vector based COVID-19 vaccines [215–217]. In US, 6 women (ages 18-48 years) were diagnosed with cerebral venous sinus thrombosis during 6-13 days after vaccination with Janssen vaccine [218]. In comparison to non-vaccinated population, the ChAdOx1-S recipient cohort showed 1.97 folds higher venous thromboembolic events morbidity and 20.25 folds higher cerebral venous thrombosis [219]. During phase III clinical trial of Sputnik V, a candidate developed deep vein thrombosis [220].

Merits and Demerits — Availability of multiple viral vectors and ease of manipulation makes this platform valuable and flexible for the vaccine development. This system has the ability to express any antigen *via* genome manipulation and can be utilized for the administration of large DNA sequences in the viral genome. These two advantages make it ideal modality for the production and development of wide variety of vaccines. Furthermore, the administration of genetic material as an antigen is the key advantage of these vaccines, which helps in production and processing of antigen in a suitable manner. The utilization of host antigen from naturally infected patients is mostly preferred in the development of these vaccines as the antigen from other sources (viz., bacterial system) may cause difficulties and express differentially in humans. As the antigen belongs to the natural source, these vaccines are administered without any additive components making it an effective platform for pandemic situations like COVID-19 where huge production is required for the global vaccination [221–223].

In spite of the numerous advantages of viral vector-based vaccines, a number of challenges limit its effective use and several key points need to be examined during the development and production phases. The viral vectors involved are genetically modified organisms, can be hazardous for human and environment, and to regulate this, European

regulatory agencies and FDA has published guidelines to evaluate the potential risks on humans and environment. Additionally, these vaccines have probable threat of genome integration which may lead to genomic mutagenesis or even development of cancer. Such concerns can delay the development of vaccines although the need is quite high such in the pandemic situation. Furthermore, every viral system requires distinct manufacturing facilities results in increase in production cost. In addition, as the viruses can go through recombination during the manufacturing process, a special care towards cell culture must be taken and unintentional contamination with harmful microorganisms must be avoided [221–223].

If a person is previously exposed to a similar type of virus or viral vector (naturally or through vaccination), the immunity stored in the individual is in the form of memory immune cells, collectively termed as a pre-existing immunity. This preexisting immunity against viral vector based vaccines is a major challenge observed during the vaccination. Post-vaccination, these pre-existing memory immune cells recognizes the viral components or viral vectors and gets activated which leads to limited effectiveness and immune responses. Human adenovirus serotype 5 (Ad5) is well characterized and enormously studied among all the adenovirus vectors. Recently, most of the global population is studied to have natural pre-existing immunity against Ad5. The vaccination potential was found to be low in mice and non-human primates having such pre-existing immunity against Ad5. The presence of neutralizing antibodies prevents the vectors for transducing target cells while the memory T-cells expel out the transduced cells that leads to the reduction in vaccination potential and efficacy. To overcome this problem, viral vector from other adenovirus serotype (Ad35, Ad11 and Ad26) is selected based upon their low prevalence. They are less immunogenic than Ad5 but effective for the vaccine development. Alternatively, Ads from chimpanzee, cattle, and pig species is also preferred for vaccination [224,225].

Storage — It is recommended to keep lyophilized form for long term storage and liquid form for short term storage at 2-8°C [226].

2.2. Whole virus vaccines

Second and most effective class of vaccines in which inactivated or attenuated live virion particles were used, known as whole virus-based vaccines [13,227]. These vaccines are of two types discussed below:

2.2.1. Inactivated vaccines

Inactivated vaccines consist of whole virions inactivated chemically or by radiation, hence inactive in nature. They consist of all immunogenic components of original parent virions but are in an inactive state. They are much safer than live attenuated vaccines if the inactivation is properly done. These vaccines have a strong immunogenic response. Structures of immunogenic epitopes are completely deformed due to the inactivation of virion particles. SARS-CoV-2 inactivated vaccines are documented to induce lung pathology due to eosinophilic storm. Because of these disadvantages, SARS-CoV-2 inactivated vaccines are not that much attractive approach for vaccine development [158,228].

The production of inactivated vaccines is relatively easier than others and is capable to induce a strong immunogenic response against the multiple epitopes of the viral surface [229]. A strong eosinophilic pro-inflammatory pulmonary response as a major challenge is recently documented, due to the SARS-CoV inactivated vaccines. It is also documented that vaccination of SARS-CoV N protein component increases the pulmonary immune-pathological changes. Hence, it is mandatory to find novel methods through which pathological response of anti-N inactivated vaccines can minimize and protein S specific immune response can be enhanced. The safety of inactivated vaccines must be investigated before their clinical applications [230–233].

In preclinical studies, these vaccines against SARS-CoV-2 infection are documented to produce a huge amount of neutralizing antibodies. These neutralizing antibodies are highly specific to viral proteins such as S, N, and M. In *in-vivo* experiments in mice, antibodies against proteins N and S were found higher than that of M protein. It may suggest that epitopic polypeptides from protein N/S are key targets for the development of recombinant vaccines against SARS-CoV-2 [228,234].

Production — Inactivated vaccine, also known as killed vaccine consists of dead pathogenic particles which have been rendered incapable of producing infection related illness by chemical treatments, heat or gamma radiation. These vaccines were first introduced to combat cholera, typhoid and plague during late 1800s and early 1900s. At present, inactivated vaccines have been designed against polio, rabies. influenza, pertussis and hepatitis A. Immunogenicity exerted by these vaccines is generally weak, therefore the immune response is elicited by aid of immunological adjuvants and vaccination program includes multiple booster doses [235–237].

Mechanism — Depending upon the coupled adjuvant, inactivated vaccines can stimulate both humoral and cellular immunological responses. Once the vaccine is injected, the APCs recognize and take the antigen, transporting it to draining lymph nodes where T cells are activated leading to specialized adaptive responses along with development of immunological memory against future infection [238–240].

Delivery — IM administration is the most preferable route for delivery as these vaccines are usually coupled with adjuvant, may lead to local side effects like redness, swelling and pain [236,241].

Clinical status of inactivated vaccines against SARS-CoV-2 — Clinically approved inactivated vaccines are developed against typhoid [242], cholera [243], hepatitis-A virus [244], plague [245], rabies [246], influenza [247,248], and polio [249,250]. SARS-CoV-2 inactivated vaccines are in various developmental phases: Covi Vax (National Research Centre Egypt), Adjuvanted Inactivated Vaccine (The Scientific and Technological Research Council of Turkey), Recombinant NDV Vectored Vaccine (Laboratorio Avi-Mex), and Koçak-19 Inaktif Adjuvanlı COVID-19 Vaccine (Kocak Farma) presently in phase-I clinical trial; KoviVac (Chumakov Center) is in phase-II; vaccines QazVac (Kazakhstan RIBSP), Inactivated (Vero Cells) (Chinese Academy of Medical Sciences), Covaxin (Bharat Biotech), COVID-19 Inactivated Vaccine (Shifa Pharmed Industrial Co), BBIBP-CorV (Sinopharm (Beijing)), Inactivated (Vero Cells) (Sinopharm (Wuhan)), CoronaVac (Sinovac), ERUCOV-VAC (Health Institutes of Turkey), VLA2001 (Valneva), SARS-CoV-2 Vaccine (Vero Cells) (Minhai Biotechnology Co), KD-414 (KM Biologics Co Ltd) and FAKHRAVAC (MIVAC) (Organization of Defensive Innovation and Research) are in a phase-III clinical trial. Covaxin (Bharat Biotech), BBIBP-CorV (Sinopharm (Beijing), Vero Cells (Sinopharm (Wuhan)), CoronaVac (Sinovac), KoviVac (Chumakov Center), QazVac (Kazakhstan RIBSP), Co SARS-CoV-2 Vaccine (Vero Cells) (Minhai Biotechnology), FAKHRAVAC (MIVAC) (Organization of Defensive Innovation and Research) and COVID-19 Inactivated Vaccine (Shifa Pharmed Industrial Co) were already approved and in use [30]. Still, several other inactivated vaccines are in the pipeline of various development stages.

Covaxin showed significantly high GMTs and increased SARS-CoV-2 IgG seropositive (194.3) than the seronegative ones (118) and increased GMTs for S1 protein (9742), RBD (4124), and N protein (4161) with 77.8%, 63.6%, and 93.4% efficacy against symptomatic, asymptomatic, and severe COVID-19 cases respectively. It has been approved

for use in 12 countries and clinical trials are registered for Phase-I(2)(2), Phase-II(3)(3); and Phase-III(2)(2) [251,252]. The Beijing and Wuhan centers of Sinopharm developed two inactivated viral vaccines: BBIBP-CorV and Vero Cells (also called WIBP-CorV). BBIBP-CorV showed prominent humoral response and production of 100% neutralizing antibody titters with an overall efficacy of 79% and has been approved for use in 72 countries [253] whereas WIBP-CorV approved in China and Philippines showed 99.3% seroconversion rate and 94.5 GMT with 72.8% and 100% efficacy against symptomatic and severe cases, respectively [253]. BBIBP-CorV clinical trials are underway in 10 countries for Phase-I(3)(3), Phase-II(8)) and Phase-III(8)(8) [254]. WIBP-CorV is registered for Phase-I(1), Phase-II(2)(2); and Phase-III(5)(5) [255]. Upon CoronaVac vaccination, seroconversion rate of S1-RBD IgG and neutralizing anti-S1-RBD in adults (18–59 years old) was found to be 95.6% and 96%, whereas in elderly (60 years and older) it was 87.5 % and 100%, respectively with an overall efficacy of 84% and 50.7% and 83.7% against symptomatic and mild cases respectively. This vaccine has been approved for use in 47 countries and clinical trials are registered in Phase-I(5) Phase II(11) and Phase III(11) [256–258]. KoviVac is taken twice intramuscularly over two weeks and has been approved for emergency use in Russian Federation with one ongoing trial registered in Phase-I and II [259,260]. QazVac developed by Kazakhstan RIBSP induced Th1 response and showed an increase in neutralizing antibody titres by 4-fold. It has been approved in Kazakhstan and Kyrgyzstan, and has one trial registered in Phase-I & II (NCT04530357) and phase-III (NCT04691908) trials [261,262]. Co SARS-CoV-2 Vaccine (Vero Cells) by Minhai Biotechnology Increased T-cell and positive IFN-γ immunospot responses. GMTs increased from 29·3 to 49·1 (Day 0-14) and 100·2 to 131·7 (Day 0-28) and RBD-IgG 605·3 to 1169·8 (Day 0-14) and from 1496·8 to 2485·5 (Day 0/28). Co SARS-CoV-2 vaccine has been approved in China and Indonesia and two trials have been registered in Phase-I(2), Phase-II(2) and Phase III(1) [263,264]. FAKHRAVAC (MIVAC) (Organization of Defensive Innovation and Research) and COVID-19 Inactivated Vaccine (Shifa Pharmed) is approved by Iran as dual dose vaccine which could be injected intramuscularly 21 and 28 days apart. Both vaccines are still in clinical trials; therefore, the efficacy data has not been published yet. COVID-19 Inactivated Vaccine showed the presence of neutralizing antibodies in 93.5% of the vaccinated individual. Presently, for FAKHRAVAC only one clinical trial has been registered in each Phase-I Phase-II and Phase-III [265,266]. On the other hand, Shifa COVID-19 Inactivated Vaccine has been registered for Phase-I(3), phase-II(2) Phase III(1) [267,268].

Adverse effects — Adverse reaction of mild and severity was reported in 44.2% and 41.7% participants, who received WIV04 or HB02 vaccine, respectively. Most common side effect was pain at injection site followed by headache [253]. Common adverse effects related to vaccination with COVAXIN include pain and swelling at the site of injection, fever, headache, malaise, vomiting, nausea and skin rashes. After vaccination with COVAXIN, Herpes zoster reactivation was reported in a 60 years old male with underlying hypertension and type II diabetes mellitus condition [269]. A CoronaVac recipient reported urticaria in phase I clinical trial [258] and 82 years old female developed petechial rash one day after receiving CoronaVac vaccine as a hypersensitivity reaction [270].

Merits and demerits — These vaccines have increased stability than live pathogens which aids in its storage and transportation. Also, the loss of pathogenicity avoids the risk of reactivation of the vaccine as virulent form and can be administered in immunocompromised groups. Lack of robust immunological responses is the major setbacks of these vaccines, and the maintenance of proper defense responses require extra doses and adjutants. But over time antibody titer level in the recipients can reduce. Moreover, culturing the pathogen during manufacturing requires good biosafety facility and makes the whole process of vaccine development lengthy [238–240,271].

Storage — Vaccine must not be frozen and should be kept in a sealed setup at temperature between 2°C and 8°C [240].

2.2.2. Live attenuated vaccines

Live attenuated vaccines are live, weak, and deleted or mutated pathogenic components of the viral genome. They consist of full live parent virion but are in the attenuated state. These vaccines have strong immunogenicity and are popular for controlling several types of infections such as Mumps, Measles, Polio (Sabin), Yellow Fever, Rota virus, Varicella, Bacillus Calmette–Guerin (BCG), and Rubella. Still, these vaccines have a high risk for possible reversion of virions from attenuated to virulent state. This may create a serious problem in an immune-compromised person. Due to the serious disadvantages, the biosafety of these vaccines should be checked before proceeding to the clinical trials [272].

Production — Live attenuated vaccines are developed using live pathogens having extremely little to no virulence. The pathogens are weakened to form harmless and non-virulent forms

which are capable of felicitating quick and potent immunological responses for long-term. Pathogen attenuation can be carried out by introduction of evolutionary mutation and reduced selection pressure *via* continuous passages in foreign host systems like live animals, cell lines and embryonic eggs. Moreover, the attenuation can also be achieved by reverse genetics. These vaccines stimulate the host immune system to generate antibodies and memory cells against specified pathogens. Today, the commonly known live attenuated vaccines have been designed against influenza, rubella, measles, yellow fever and mump [273–275].

Delivery — These vaccines can be delivered *via* subcutaneously, intradermally or through nasal or oral passage [272,276].

Mechanism — Live attenuated vaccines function through induction of macrophage based cellular immunity and activation of CD8+ cytotoxic T cell responses, followed by development of antibody based humoral responses. As long as the population of these cells is maintained inside the host, a long-lasting immunity can be archived [276–278].

Clinical status of live attenuated vaccines against SARS-CoV-2 — These vaccines were produced by weakening the infectious organism which can be able to propagate and capable to produce protective immunogenic responses. These attenuated organisms are not capable to cause any disease. These vaccines are known to induce both innate as well as adaptive immunity with long-lasting immunogenic memory. These vaccines can be produced in less time and at a minimum cost. Thus, these vaccines are better and ideal to respond against lethal coronavirus outbreak [279–282]. Live-attenuated vaccines COVI-VAC (Codagenix Inc) is currently in the early stages (phase-I) of development. Still, none of these two live attenuated vaccines are approved yet even for emergency use [30].

Merits and demerits — Live attenuated COVID-19 vaccine can closely mimic the real-life infection and can stimulate long-term humoral and cellular immunity in the vaccinated people. Vaccination is cost effective, do not require frequent boosters, and can be achieved by single dose. In rare scenarios, attenuated pathogens may revert to virulent form. One such instance was the gain of virulence of poliovirus in oral polio vaccine (OPV). Due to such risks, individuals with immune system disorders and pregnant/breast-feeding women are advised not to take this vaccine. It has been reported that yellow fever and varicella based live vaccines lead to adverse complications in fetuses and infants. Moreover, maintenance of live pathogens requires high-end facilities [238,283].

Storage — It is recommended to store the live attenuated vaccine in a sealed container at 2-8°C. This temperature slows-down the pathogen's metabolism and replication [276,283].

3. Adjuvants used in anti-SARS-CoV-2 vaccines development

Most of the vaccine types are under development stages by using the several platforms according to their reactivity and specificity to develop immune responses. The addition of adjuvants to vaccines is also a potential platform to improve the efficacy and duration of their immunological responses of the vaccine. Additionally, adjuvants are also preferred to reduce the concentration of antigen used and the number of immunizations required for their protective efficacy. It also makes the vaccines much cost effective and therapeutically potent. Moreover, the incorporation of adjuvants for development of subunit and certain inactivated vaccines is preferred due to the occasional lack of specific immune responses. Incorporation of adjuvants will improve the magnitude, direction and specificity of immune responses [284].

Various types of adjuvants such as aluminum salts (2% alhydrogel), STING agonists (CF501), manganese (nanoMn), oil/water emulsions (MF59, AS03), TLR agonists (LR1/2, TLR3, TLR4, TLR7/8), cationic nano-carriers (Chitosan, PEI, DOTAP), matrix-M1 and Advax-SM (Advax™ & CpG55.2) are preferred in the development of anti-SARS-CoV-2 vaccines (Table 3). 2% alhydrogel is preferred to be used as an adjuvant in both inactivated as well as RBD subunit vaccines to activate the pro-inflammatory NLPR3 pathway and T-helper 2 cell responses [258,285]. CF501 is a STING agonist preferred to use in RBD-Fc region protein subunit vaccines to activate both cellular and humoral immune responses [286,287]. NanoMn is also used in RBD protein vaccines to enhance the production of cGAMP and its binding with STING [288,289]. Furthermore, MF59 and AS03 oil in water emulsion adjuvants are studied to be used in S-protein based vaccines to enhance the dendritic cell recruitment, CD4+ T cell priming and humoral immunity [290,291]. TLR agonists are also preferred to use in both subunit and inactivated vaccine development to induce IFN, pro-inflammatory cytokines and chemokines [292–295]. Nano-carriers such as chitosan, PEI and DOTAP are also used as adjuvants in RBD subunit vaccines to activate the cytotoxic CD8+ and CD4+ T cells [296]. Adjuvant matrix-M1 is used in trimeric S-protein vaccines to activate both cellular and humoral immunity [155,297]. Adjuvants Advax™ and CpG55.2 are preferred to be used in S protein vaccines that activates CD8+ dendritic cells and robustness of T-cell based response [298]. Thus, the use of adjuvants is

important in the development of various anti-SARS-CoV-2 vaccines to promote the immune specificity and durability.

4. Can vaccine administration *via* intramuscular (IM) route prevent virus transmission?

Majority of the SARS-CoV-2 vaccines available for mass vaccination are administered intramuscularly (IM) and provide significant induction of IgG-neutralizing response against viremia and disease severity in systemic circulation. The prime effects of these vaccines are prevention of disease related morbidity and mortality, but they do not confer mucosal immunity, a crucial inhibitor of viral transmission. SARS-CoV-2 mostly enters through mucosal surface of upper respiratory tract (nasopharynx), whereas IM vaccination mainly stimulates immunity in lower tract and does not transport sufficient neutralizing antibodies to the mucosal lining or induce significant mucosal immunity by elicitation of secretory mucosal polymeric IgA, IgG and tissue-resident memory T cells (T_{RM}). Mucosal IgA can inhibit viral load and lower the chances of disease severity by neutralizing the invading viral particles and protect the uninfected epithelial cells from infection. In addition, IgA facilitate functionality of targeted effector by Fcα-receptor cross linking and enhances antibody affinity by polymeric IgA [299]. It has been documented that translocation of circulating polymeric or non-polymeric IgA is relatively low in secretion [300] which might make intramuscularly induced systematic antibodies less dominant in mucosal layers. Circulating IgG and cytotoxic T cells induced by IM delivery stimulates inflammation by phagocytic and through complement activation which tackles the propagation of severe SARS-CoV-2 pathology in terminal lungs airways by removing already infected cells but does not have potent effect on infection prevention. Mucosal IgA can be elevated by protein-subunit based vaccines delivered intranasally, but induction of mucosal CD8+ T_{RM}, requires locally produced antigen presented by MHC-I of stromal and dendritic (CD103+) cells. As these T_{RM} stays in close vicinity of viral doorways like respiratory airways and epithelium, upon secondary infection, they can quickly react with humoral and cellular responses [299]. It was reported that Ad26.COV2.S vaccine failed to induce production of IgG and IgA antibodies in recipient saliva [301]. Also, IM delivery of BNT162b2 vaccine enhance the anti-spike immunoglobin A (1&2) and G (1, 2 & 3) in serum but only IgG antibody in saliva. However, after a week or two of second dose, the level of salivary IgG enhanced and IgA was detected in few individuals [302]. For conferring IM vaccination associated

mucosal effector induction, translocation of immunoglobins and their kinetics in circulation and secretion still requires advanced research. To induce the mucosal immunity for inhibition of viral transmission, along with induction of systematic immunity; intranasal vaccines should be designed which can provide immunization of nasopharynx-associated lymphoid tissue (NALT) and upper respiratory tract [300,303,304].

To overcome the severe effects of SARS-CoV-2 pandemic, all the vaccines available have been authorized for emergency use. The clinical trial of these vaccines majorly focused on healthy adults and efficacy drawn does not include cohorts with complicated medical conditions. Children, pregnant/nursing women and individuals with immune system disorders are extremely vulnerable to infection related serious outcomes, therefore special attention must be paid for vaccination of these groups [305]. In the next section, vaccination possibilities for special group of individuals have been discuses.

5. Vaccination of medically distinct population

In initial clinical trials of SARS-CoV-2 vaccines, specials cohorts like pregnant and breast-feeding women, children and adolescents, immune-compromised and autoimmune population have not been included. Such group presents higher infection related morbidity, and the approved vaccines may also react adversely. Specialized vaccines are essential for achieving significant vaccination rate of these groups [306].

5.1. Pregnant and breast-feeding women

In comparison to non-pregnant population, pregnant women do not show any major difference in infection transmission rate, however they are at a higher risk of severe infection related illness. Occurrence of perinatal complications like placenta thrombosis, placentitis, premature births, miscarriages and stillbirths were reported, and infants are commonly referred to the neonatal care unit. Maternal gestational diabetes, pre-eclampsia and PTSD (post-traumatic stress disorder) were also observed [305,307]**.** CDC reported that in the US, 0.44% pregnant SARS-CoV-2 infected women were transferred to ICU and 0.11% died [308]. Another study showed that infected pregnant women in ICU had 70% increases in mortality risk, received ECMO (extracorporeal membrane oxygenation) and were on invasive ventilation [309]. *In vivo,* preclinical investigation of COVID-19 vaccines on pregnant and breast-feeding women has not

raised any safety alarm, but currently no vaccines have been registered for pre-marketing trial in this cohort [310].

BNT162b2 mRNA vaccine is currently under phase III clinical trial (NCT04754594) and Ad26.COV2.S vaccine (registered as NCT04765384) is in phase II trial and is actively recruiting participants. In an observational study in Israel, BNT162b2 showed 96% overall effectiveness, 97% against symptomatic infection and 89% against hospitalization in pregnant women [311]. In another study, vaccine effectiveness was found to be 78% and no severe adverse effect was reported. Less than 0.1% individuals showed fatigue, headache, stomachache, dizziness and rashes. Major limitation of these studies is the lack of information regarding perinatal effects [312]. Development of prominent maternal IgG antibody upon vaccination with mRNA vaccines (Pfizer-BioNTech and Moderna vaccines) and subsequent translocation into placenta has also been reported [313]. In another cohort study, breast milk samples of Pfizer-BioNTech vaccinated individuals (2 doses) were analyzed for presence of antibodies. Virus specific IgA and IgG antibody was secreted significantly for 6 weeks post vaccination [314].

WHO recommends vaccination in pregnant women when the potential risk associated outweighed by vaccination benefits in events like high risk of COVID-19 infection and presence of pregnancy related co-morbidities [315]. In UK Pfizer-BioNTech or Moderna mRNA vaccines are specially recommended by Royal College of Obstetricians and Gynaecologists, no adverse effect was associated in 275,000 vaccinated pregnant women [316].

5.2. Children/adolescents

Cohort based study showed low SARS-CoV-2 transmission in children as compared to adults. 234 children aged between 0 to 10 belonging to populations showing 71.5% and 85.9% infection**,** were found to be COVID-19 negative even after being in close contact with positive family members [317–319]. On event of infection, children were asymptomatic or had mild symptoms like weakness, fever and cough [320,321]. In US, the preliminary cumulative rates of COVID-19 related hospitalization as of Feb 05, 2022 was 913.5/100,000 adult population (aged 18 and above) which was significantly higher than 166.3 in 0 to 4 years old, 49.4 in 5 to 11 years old and 110.3 in 12 to 17 years old children [321]. Moreover, preliminary IgG-SARS-CoV-2 seroprevalence investigation reported only 1.3% in 0 to 5 year old group [322]. In comparison to children/adolescents, neonates and infants are more susceptible to severe induction related

diseases like Kawasaki-like illness, pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 virus (PIMS-TS) and mortal shock [323,324].

Currently, 13 trials in phase I, 31 in phase II and 26 in Phase III have been registered for clinical investigation of SARS-CoV-2 vaccine Vero Cells, Sf9 Cells, CHO Cells, BBV152, inactivated vaccine (Sinovac), Ad5-nCoV-IH, 9vHPV, mRNA-1273, MVC-COV1901, BNT162b2, CoronaVac, Gam-COVID-vac M, SCB-2019, LV-SMENP-DC, aAPC and COVAXIN for children and adolescents. FDA approved Pfizer-BioNTech vaccine for 5-11 years old children based on 90.7% efficacy is comparable to adults. Moreover no adverse effect was observed in 3100 enrolled children [325]. Later the vaccine was approved in 12-15 years old participants showing 100% efficacy as well as 16 years and above showing 94.6% efficacy. Post authorization adverse effects included pain at the site of injection, diarrhea and hypersensitivity reactions like anaphylaxis, rashes, angioedema, and pruritus [325].

At this moment, clinical evaluations of 8 trials are registered in phase I, 18 in phase II and 10 in phase III. The vaccines included in these trials are Nanocovax (NCT04683484, NCT04611802), BNT162B2 (NCT04895982, (NCT04761822, NCT04368728, NCT04713553, NCT04816643, NCT04800133), VLA2001 and VLA2101 (NCT04956224), COVAXIN (NCT04918797, NCT04471519), Ad5-nCOV (NCT04566770, NCT04916886), UB-612 (NCT04773067), Spikevax (NCT04761822, NCT04796896, NCT04649151), Vero cells (NCT04917523, NCT04884685, NCT04551547), CHO Cell (NCT04869592), aAPC Vaccine (NCT04299724), MVC-COV1901 (NCT04951388), LV-SMENP (NCT04276896) and Gam-COVID-Vac (NCT04954092) *(ClinicalTrials.gov).*

5.3. Immune system disorder (immunodeficiency & autoimmune/auto-inflammatory diseases)

Observational studies have reported that cohorts with underlying conditions like immunodeficiency and autoimmunity are potentially vulnerable to severe COVID-19 associated diseases. Individuals with hematologic malignancies, solid tumor, organ/cell transplant, HIV/AIDS, allergy, autoimmunity, and primary/secondary immunodeficiency are at higher risk [326]**.** Moreover, Therapeutic approaches like chemotherapy under COVID19 effect have shown increased mortality of 30 days [327]. Metadata analysis of 300000 autoimmunity-autoinflammatory patient showed that, the intake of steroids considerably increases the

COVID19 infection in this group [328]**.** Individuals with immune system disorder should avoid live virus based vaccines and must opt for subunit and inactivated vaccines [329].

In a study, 46% individual among 658 solid organ transplant enrolees, vaccinated with SARS-CoV-2 mRNA vaccines showed absence of humoral antibody response [330]. BNT162b2 and ChAdOx1 vaccinated individuals with haematological malignancies and solid organ tumors or recipient of immunosuppressive treatment had significantly low spike protein antibodies in partially vaccinated (single dose) candidates than in fully vaccinated ones (dual doses) which stresses the efficacy of early administration of second dose in these groups [331]. The humoral response induced by BNT162 vaccine showed lack of IgG titres production in dialysis and kidney transplant [332]. Similar outcome was observed in case of lung transplant recipients, where virus specific IgG level was studied in 33 patients with history of COVID-19 infection and 48 who were vaccinated with BNT162 vaccine. 85% of the participants with prior infection showed presence of humoral antibody response but it was completely absent in the vaccinated group. The lack of humoral immune response from vaccine could be result of immunosuppressive drugs involved in the treatment of this cohort [333]. Major challenges in vaccination are its effect on other prescribed treatments and adverse effects. In patients with auto-immune/inflammatory disorders, vaccine may lead to elevated stimulation of stabilized illness. Among 491 BNT162b2 vaccinated autoimmune and inflammatory rheumatic disease patients 1.2% showed Herpes zoster reactivation [334].

However, the lack of clinical results regarding vaccine efficacy and safety does not outweigh the fatal COVID-19 linked complication of these individuals and makes them top priority of COVID-19 vaccination drive. The efficacy of BNT162B2 and ChAdOx1 after second dose considerably increases to 73% and 74.6%, respectively and efficacy of third dose is currently being investigated [335]. According to Australasian Society of Clinical Immunology and Allergy (ASCIA), non-live attenuated vaccines *viz.*, mRNA-based COVID-19 vaccines, BNT162B2 (Pfizer/BioNTech) and Spikevax (Moderna) and viral vector COVID-19 vaccine ChAdOx1 (AstraZeneca/Oxford) are safe for individuals with immunodeficiency, autoimmunity, auto-inflammation and allergy [336].

A total of 4927 immune-deficient individuals are currently enrolled in six clinical trials (*viz.*, 1 in phase II, 2 in phase III & IV, and 1 with unknown status). These vaccines include two mRNA vaccines: BNT162B2 (NCT04895982, NCT04780659) and Spikevax (NCT04806113, NCT04805125, NCT04847050), an Adenovirus vector based Covishield

vaccine (NCT04794946) and whole virus inactivated vaccine CoronaVac (NCT04754698). All enrolled candidates are ≥18 years of age and associated conditions include primary/secondary immunosuppressive disorders, HIV/AIDS, organ transplantation, solid and hematological malignancies, liver cirrhosis and rheumatic diseases *(ClinicalTrials.gov).*

Recently, the cellular and humoral responses of BNT162b2 vaccine is assessed in patient groups with rheumatoid arthritis receiving treatment with methotrexate (synthetic) and TNF blockers (biologic). TNF blocker recipient group shows strong antibody responses in more than 90% patients while methotrexate recipient group shows adequate response in 62.2% patients. It occurs due to the prevention of CD8+ T-cell activation in the methotrexate treated arthritis patients after vaccination. Further, much study is needed to be performed to confirm these results and to make safer vaccination of arthritis patients [337].

However, the inflammatory bowel disease (IBD) patient's treatment along with anti-SARS-CoV-2 vaccination is also important to study. Recently, in a study IBD patient groups treated with 6 different drugs (*viz.*, thiopurines, tofacitinib, thiopurine + infliximab, ustekinumab, infliximab and vedolizumab) and two doses of vaccines (*viz.*, AstraZeneca, BNT162b2 and mRNA1273). Immediately, after the second dose of vaccine the antibody concentration were measured. Vaccinated IBD patient groups treated with different drugs such as Infliximab, infliximab + thiopurine and tofacitinib shows lower production of anti-SARS-CoV-2 spike protein antibody while ustekinumab, thiopurine and vedolizumab treated IBD patients shows no such significant change in antibody concentration. This study suggests that Infliximab, Infliximab + thiopurine and tofacitinib recipient IBD patient groups need 3rd booster dose to maintain the anti-SARS-CoV-2 antibody concentration [338].

Anti-rheumatic drugs (DMARDs) are one of the possible treatment options for the immuno-inflammatory diseases. SARS-CoV-2 infection shows complications in DMRD (biologic) treated individuals. Vaccination of this population may reduce such serious complications. Recently, a clinical study was performed to evaluate the immune responses in TNF inhibitor (adalimumab) or IL-17A inhibitor (secukinumab) receiving spondyloarthritis patient groups receiving dual doses of BNT162b2 vaccine. The high seroconversion rate was observed in both the test groups. The huge production of CD4+ and CD8+ T-cells but no such significant change is observed in the level of reactive T-cells between the test groups. Thus, this study concluded that both the biologic DMRDs are not able to affect the cellular as well as humoral immune responses of BNT162b2 vaccinated spondyloarthritis patients. Thus, more

clinical trial based study is required to evaluate and assess the vaccine safety and efficacy in these type of immunosuppressive diseases [339].

5.4 Inborn error of immunity

Primary immunodeficiency or "inborn error of immunity" (IEI) as phrased by the International Union of Immunological Societies (IUIS), are germline disorders which alter optimal performance of immune system making individuals susceptible to infections, allergies autoinflammatory disorders, autoimmune diseases and malignancies [340–342].

IEI patients vs SARS-CoV-2 infection — SARS-CoV-2 infection in immunocompromised patients is associated with elevated severity and ICU admission. Several case studies have reported that mortality and morbidity through COVID-19 related complications like chronic lung/liver diseases and prophylactic antibiotics were raised in young IEI patients in comparison to the general cohort [343–346]. Another study on 4718 Iranian PID patients showed an increase in COVID-19 infection and mortality rate by 1.23 and 10 folds respectively [347]. It has been reported that some IEI patients with deficient antibodies had considerably robust humoral and T cell-based responses against SARS-CoV-2 spike and nucleocapsid proteins [348]. Another study showed that SARS-CoV-2 infected IEI individuals had 4 months long viral shedding and T cell activity against the virus [349].

Vaccination immune Responses — To develop suitable vaccine for IEI individuals it is important to assess response of existing COVID-19 vaccines in the PID population. Various studies have been conducted to evaluate the immunological effect of RNA based vaccines (*viz.*, BNT162B2, mRNA-1273) and inactive viral vaccines (*viz.*, ChAdOx1 nCoV-19, Ad26.COV2.S and Coronovac) in IEI patients [350]. A study showed that among 81 IEI individuals, 85% generated antibodies against spike protein after primary doses of AdV26.COV2.S and mRNA based vaccines, whereas lower anti-S IgG level was observed in individuals with history of rituximab medication and autoimmune polyendocrinopathy candidiasis ecto-dermal dystrophy (APECED) patients [351]. BNT162B2 vaccination led to seroconversion in 73% IEI patients which included monogenic disorder and common variable immunodeficiency (CVID). Salivary anti-S IgG level were relatively low in IEI population [352]. A survey of 505 IEI patients vaccinated with mRNA-1273 reported that in comparison to the general population, seroconversion was similar in milder IEIs but was lower in severe IEIs like

CID and CVID [353]. Various articles reported that vaccination responses associated with diverse B cell dysfunction of IEIs. Among 17 CVID patient vaccinated with BNT162B2 in cohort with normal peripheral and switched B cell population showed usual serologic activity but in individuals with deficient switched memory B cells showed lower serologic responsees were observed. Moreover, serologic activity was totally absent in patients lacking peripheral B cells [354] (Table 4). Overall, post vaccination, serological responses are apparent in IEI populations but the long-term efficacy of the vaccines is still uncertain. Few studies showed comparable decrease in antibody titer in IEI and normal population [350].

Vaccinations safety — Low immunological efficiency of IEI patients requires extra attention at the safety of COVID-19 vaccines. An article reported that IEI patients vaccinated with mRNA vaccine had higher reactogenicity in comparison to the general population where they observed common symptoms include mild myalgias, fever and fatigue [350]. Among, 130 individuals with various autoinflammatory conditions, vaccinated with BNT162B2 or ChAdOx1-S, no severe adverse reaction was observed without any significant inflammatory flare [355].

To reduce the severity, mortality and hospital admissions, total public vaccination is of utmost importance, and this could not be achieved without the vaccination of individuals with above-mentioned underlying complications. To date, special clinical investigation of vaccines for such groups is still under progress but not enough data is available to determine the effectiveness of approved vaccines among these cases [306]. Nevertheless, some vaccines discussed above have been approved but further examination of these vaccines is still required for safety issues in these special groups.

6. CONCLUDING REMARKS

COVID-19 pandemic is a worldwide threat with non-availability of specific treatment. In this review, we have discussed the clinical development of vaccine candidates. Ongoing vaccine development and status of many approved vaccines after successful clinical trials and various classes of vaccines which are still in progress. Here, we have highlighted the clinical status of more than a hundred vaccines. Most of the vaccines are still in the various stages of clinical trials and a few of them are approved by respective regulatory authorities for their clinical use. So, researchers and clinicians will have to put more effort and attention into clinical trial

limitations. Moreover, the investors and pharmaceutical industries must come together for the advancement of specific and efficient vaccines for children and adults.

Abbreviations

COVID-19: Coronavirus disease of 2019; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus-2; WHO: World Health Organization; MERS-CoV: Middle East respiratory syndrome coronavirus; N: Nucleocapsid; S: Spike; M: Membrane; E: Envelope; ADRP: ADP-ribose-1"-phosphate; MERS-RBD: Middle east respiratory syndrome-receptor binding domain; VLP: Virus-like particles; BCG: Bacillus Calmette Guerin; RNP: Ribonucleocapsid; K48: Lys-48; K63: Lys-63; IRF3: Interferon regulatory factor-3; mRNA: Messenger ribonucleic acid; Cryo-EM: Cryo-electron microscopy; and nm: Nanometer.

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Authors' contributions

Conceived the idea, review structure and writing: SK, MB, AA, PG and MKG. Revised the manuscript: PG, MB, SK and MKG. All authors have read and agreed to the final draft of the manuscript.

Conflicts of interests

There is no competing and conflict of interests.

REFERENCES

1. Coronavirus disease (COVID-19) – World Health Organization. Accessed August 28, 2021. https://www.who.int/emergencies/diseases/novel-coronavirus-2019

2. Van der Hoek L. Human coronaviruses: what do they cause? *Antivir Ther*. 2007;12(4 Pt B):651-658.

3. COVID-19 Map - Johns Hopkins Coronavirus Resource Center. Accessed August 6, 2021. https://coronavirus.jhu.edu/map.html

4. Brivio E, Guiddi P, Scotto L, et al. Patients living with breast cancer during the coronavirus pandemic: the role of family resilience, coping flexibility, and locus of control on affective responses. *Front Psychol*. 2021;11:3711.

5. Masiero M, Mazzocco K, Harnois C, Cropley M, Pravettoni G. From individual to social trauma: sources of everyday trauma in Italy, the US and UK during the COVID-19 pandemic. *J Trauma Dissociation*. 2020;21(5):513-519.

6. Velavan TP, Meyer CG. The COVID-19 epidemic. *Trop Med Int Health TM IH*. 2020;25(3):278-280. doi:10.1111/tmi.13383

7. Rosa SGV, Santos WC. Clinical trials on drug repositioning for COVID-19 treatment. *Rev Panam Salud Pública*. 2020;44:e40.

8. Abdelghany TM, Ganash M, Bakri MM, Qanash H, Al-Rajhi AMH, Elhussieny NI. SARS-CoV-2, the other face to SARS-CoV and MERS-CoV: Future predictions. *Biomed J*. 2021;44(1):86-93. doi:10.1016/j.bj.2020.10.008

9. Khubchandani J, Sharma S, Price JH, Wiblishauser MJ, Sharma M, Webb FJ. COVID-19 vaccination hesitancy in the United States: a rapid national assessment. *J Community Health*. 2021;46(2):270-277.

10. 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):1-23.

11. Vigant F, Santos NC, Lee B. Broad-spectrum antivirals against viral fusion. *Nat Rev Microbiol*. 2015;13(7):426-437.

12. Andreadakis Z, Kumar A, Román RG, Tollefsen S, Saville M, Mayhew S. The COVID-19 vaccine development landscape. *Nat Rev Drug Discov*. 2020;19(5):305-306.

13. Types of Vaccines – COVID19 Vaccine Tracker. Accessed August 27, 2021. https://covid19.trackvaccines.org/types-of-vaccines/

14. Sardesai NY, Weiner DB. Electroporation delivery of DNA vaccines: prospects for success. *Curr Opin Immunol*. 2011;23(3):421-429.

15. Yu J, Tostanoski LH, Peter L, et al. DNA vaccine protection against SARS-CoV-2 in rhesus macaques. *Science*. 2020;369(6505):806-811.

16. Chandrashekar A, Liu J, Martinot AJ, et al. SARS-CoV-2 infection protects against rechallenge in rhesus macaques. *Science*. 2020;369(6505):812-817.

17. Inc IP. Inovio Accelerates Timeline for COVID-19 DNA Vaccine INO-4800. Accessed December 19, 2021. https://www.prnewswire.com/news-releases/inovio-accelerates-timeline-for-covid-19-dna-vaccine-ino-4800-301015031.html

18. Huang L, Rong Y, Pan Q, et al. SARS-CoV-2 vaccine research and development: conventional vaccines and biomimetic nanotechnology strategies. *Asian J Pharm Sci*. 2021;16(2):136-146.

19. A Williams J. Improving DNA vaccine performance through vector design. *Curr Gene Ther*. 2014;14(3):170-189.

20. Minicircle DNA vectors devoid of bacterial DNA result in persistent and high-level transgene expression in vivo - PubMed. Accessed March 2, 2022. https://pubmed.ncbi.nlm.nih.gov/12946323/

21. Walters AA, Kinnear E, Shattock RJ, et al. Comparative analysis of enzymatically produced novel linear DNA constructs with plasmids for use as DNA vaccines. *Gene Ther*. 2014;21(7):645-652.

22. Lambricht L, Lopes A, Kos S, Sersa G, Préat V, Vandermeulen G. Clinical potential of electroporation for gene therapy and DNA vaccine delivery. *Expert Opin Drug Deliv*. 2016;13(2):295-310.

23. Donnelly JJ, Wahren B, Liu MA. DNA vaccines: progress and challenges. *J Immunol*. 2005;175(2):633-639.

24. Li L, Petrovsky N. Molecular mechanisms for enhanced DNA vaccine immunogenicity. *Expert Rev Vaccines*. 2016;15(3):313-329.

25. Porgador A, Irvine KR, Iwasaki A, Barber BH, Restifo NP, Germain RN. Predominant role for directly transfected dendritic cells in antigen presentation to CD8+ T cells after gene gun immunization. *J Exp Med*. 1998;188(6):1075-1082.

26. Plasmid DNA vaccines: tissue distribution and effects of DNA sequence, adjuvants and delivery method on integration into host DNA - PubMed. Accessed March 2, 2022. https://pubmed.ncbi.nlm.nih.gov/11251382/

27. Fu TM, Ulmer JB, Caulfield MJ, et al. Priming of cytotoxic T lymphocytes by DNA vaccines: requirement for professional antigen presenting cells and evidence for antigen transfer from myocytes. *Mol Med*. 1997;3(6):362-371.

28. Iwasaki A, Torres CA, Ohashi PS, Robinson HL, Barber BH. The dominant role of bone marrow-derived cells in CTL induction following plasmid DNA immunization at different sites. *J Immunol*. 1997;159(1):11-14.

29. Corr M, Lee DJ, Carson DA, Tighe H. Gene vaccination with naked plasmid DNA: mechanism of CTL priming. *J Exp Med*. 1996;184(4):1555-1560.

30. Vaccines – COVID19 Vaccine Tracker. Accessed August 27, 2021. https://covid19.trackvaccines.org/vaccines/

31. Momin T, Kansagra K, Patel H, et al. Safety and Immunogenicity of a DNA SARS-CoV-2 vaccine (ZyCoV-D): Results of an open-label, non-randomized phase I part of phase I/II clinical study by intradermal route in healthy subjects in India. *EClinicalMedicine*. 2021;38:101020.

32. Zydus Cadila: ZyCoV-D – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/29/

33. Seo YB, Shin D, Suh YS, Na J, Ryu JI, Sung YC. Marked enhancement of neutralizing antibody and IFN-γ T-cell responses by GX-19N DNA booster in mice primed with inactivated vaccine. *bioRxiv*. Published online 2021.

34. Rauch S, Jasny E, Schmidt KE, Petsch B. New vaccine technologies to combat outbreak situations. *Front Immunol*. 2018;9:1963.

35. Wang Z, Troilo PJ, Wang X, et al. Detection of integration of plasmid DNA into host genomic DNA following intramuscular injection and electroporation. *Gene Ther*. 2004;11(8):711-721.

36. Schalk JA, Mooi FR, Berbers GA, Aerts LA van, Ovelgönne H, Kimman TG. Preclinical and clinical safety studies on DNA vaccines. *Hum Vaccin*. 2006;2(2):45-53.

37. Armengol G, Ruiz LM, Orduz S. The injection of plasmid DNA in mouse muscle results in lifelong persistence of DNA, gene expression, and humoral response. *Mol Biotechnol*. 2004;27(2):109-118.

38. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines—a new era in vaccinology. *Nat Rev Drug Discov*. 2018;17(4):261-279.

39. Schlake T, Thess A, Fotin-Mleczek M, Kallen KJ. Developing mRNA-vaccine technologies. *RNA Biol*. 2012;9(11):1319-1330.

40. Alberer M, Gnad-Vogt U, Hong HS, et al. Safety and immunogenicity of a mRNA rabies vaccine in healthy adults: an open-label, non-randomised, prospective, first-in-human phase 1 clinical trial. *Lancet Lond Engl*. 2017;390(10101):1511-1520. doi:10.1016/S0140-6736(17)31665-3

41. Al-Amri SS, Abbas AT, Siddiq LA, et al. Immunogenicity of candidate MERS-CoV DNA vaccines based on the spike protein. *Sci Rep*. 2017;7(1):1-8.

42. Muthumani K, Falzarano D, Reuschel EL, et al. A synthetic consensus anti–spike protein DNA vaccine induces protective immunity against Middle East respiratory syndrome coronavirus in nonhuman primates. *Sci Transl Med*. 2015;7(301):301ra132-301ra132.

43. Pardi N, Muramatsu H, Weissman D, Karikó K. In vitro transcription of long RNA containing modified nucleosides. *Synth Messenger RNA Cell Metab Modul*. Published online 2013:29-42.

44. Weissman D, Pardi N, Muramatsu H, Karikó K. HPLC purification of in vitro transcribed long RNA. In: *Synthetic Messenger RNA and Cell Metabolism Modulation*. Springer; 2013:43-54.

45. Gallie DR. The cap and poly (A) tail function synergistically to regulate mRNA translational efficiency. *Genes Dev*. 1991;5(11):2108-2116.

46. Parker R, Song H. The enzymes and control of eukaryotic mRNA turnover. *Nat Struct Mol Biol*. 2004;11(2):121-127.

47. Brito LA, Kommareddy S, Maione D, et al. Self-amplifying mRNA vaccines. In: *Advances in Genetics*. Vol 89. Elsevier; 2015:179-233.

48. Perri S, Greer CE, Thudium K, et al. An alphavirus replicon particle chimera derived from venezuelan equine encephalitis and sindbis viruses is a potent gene-based vaccine delivery vector. *J Virol*. 2003;77(19):10394-10403.

49. Geall AJ, Verma A, Otten GR, et al. Nonviral delivery of self-amplifying RNA vaccines. *Proc Natl Acad Sci*. 2012;109(36):14604-14609.

50. Iavarone C, O'hagan DT, Yu D, Delahaye NF, Ulmer JB. Mechanism of action of mRNA-based vaccines. *Expert Rev Vaccines*. 2017;16(9):871-881.

51. Vogel AB, Lambert L, Kinnear E, et al. Self-amplifying RNA vaccines give equivalent protection against influenza to mRNA vaccines but at much lower doses. *Mol Ther*. 2018;26(2):446-455.

52. Selmi A, Vascotto F, Kautz-Neu K, et al. Uptake of synthetic naked RNA by skin-resident dendritic cells via macropinocytosis allows antigen expression and induction of T-cell responses in mice. *Cancer Immunol Immunother*. 2016;65(9):1075-1083.

53. Granstein RD, Ding W, Ozawa H. Induction of anti-tumor immunity with epidermal cells pulsed with tumor-derived RNA or intradermal administration of RNA. *J Invest Dermatol*. 2000;114(4):632-636.

54. Carralot JP, Probst J, Hoerr I, et al. Polarization of immunity induced by direct injection of naked sequence-stabilized mRNA vaccines. *Cell Mol Life Sci CMLS*. 2004;61(18):2418-2424.

55. Kreiter S, Selmi A, Diken M, et al. Intranodal vaccination with naked antigen-encoding RNA elicits potent prophylactic and therapeutic antitumoral immunity. *Cancer Res*. 2010;70(22):9031-9040.

56. Bialkowski L, van Weijnen A, Van der Jeught K, et al. Intralymphatic mRNA vaccine induces CD8 T-cell responses that inhibit the growth of mucosally located tumours. *Sci Rep*. 2016;6(1):1-15.

57. Sahin U, Derhovanessian E, Miller M, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. *Nature*. 2017;547(7662):222-226.

58. Qiu P, Ziegelhoffer P, Sun J, Yang NS. Gene gun delivery of mRNA in situ results in efficient transgene expression and genetic immunization. *Gene Ther*. 1996;3(3):262-268.

59. Steitz J, Britten CM, Wölfel T, Tüting T. Effective induction of anti-melanoma immunity following genetic vaccination with synthetic mRNA coding for the fusion protein EGFP. TRP2. *Cancer Immunol Immunother*. 2006;55(3):246-253.

60. Aberle JH, Aberle SW, Kofler RM, Mandl CW. Humoral and cellular immune response to RNA immunization with flavivirus replicons derived from tick-borne encephalitis virus. *J Virol*. 2005;79(24):15107-15113.

61. Lutz J, Lazzaro S, Habbeddine M, et al. Unmodified mRNA in LNPs constitutes a competitive technology for prophylactic vaccines. *NPJ Vaccines*. 2017;2(1):1-9.

62. Karikó K, Muramatsu H, Ludwig J, Weissman D. Generating the optimal mRNA for therapy: HPLC purification eliminates immune activation and improves translation of nucleoside-modified, protein-encoding mRNA. *Nucleic Acids Res*. 2011;39(21):e142-e142.

63. Rozema DB, Lewis DL, Wakefield DH, et al. Dynamic PolyConjugates for targeted in vivo delivery of siRNA to hepatocytes. *Proc Natl Acad Sci*. 2007;104(32):12982-12987.

64. Pardi N, Hogan MJ, Pelc RS, et al. Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination. *Nature*. 2017;543(7644):248-251.

65. Bahl K, Senn JJ, Yuzhakov O, et al. Preclinical and clinical demonstration of immunogenicity by mRNA vaccines against H10N8 and H7N9 influenza viruses. *Mol Ther*. 2017;25(6):1316-1327.

66. Akinc A, Querbes W, De S, et al. Targeted delivery of RNAi therapeutics with endogenous and exogenous ligand-based mechanisms. *Mol Ther*. 2010;18(7):1357-1364.

67. Pardi N, Tuyishime S, Muramatsu H, et al. Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. *J Controlled Release*. 2015;217:345-351.

68. Richner JM, Himansu S, Dowd KA, et al. Modified mRNA vaccines protect against Zika virus infection. *Cell*. 2017;168(6):1114-1125.

69. Chen N, Xia P, Li S, Zhang T, Wang TT, Zhu J. RNA sensors of the innate immune system and their detection of pathogens. *IUBMB Life*. 2017;69(5):297-304.

70. Lässer C, O'Neil SE, Shelke GV, et al. Exosomes in the nose induce immune cell trafficking and harbour an altered protein cargo in chronic airway inflammation. *J Transl Med*. 2016;14(1):1-14.

71. Edwards DK, Jasny E, Yoon H, et al. Adjuvant effects of a sequence-engineered mRNA vaccine: translational profiling demonstrates similar human and murine innate response. *J Transl Med*. 2017;15(1):1-18.

72. Kowalczyk A, Doener F, Zanzinger K, et al. Self-adjuvanted mRNA vaccines induce local innate immune responses that lead to a potent and boostable adaptive immunity. *Vaccine*. 2016;34(33):3882-3893.

73. Efficient Targeting and Activation of Antigen-Presenting Cells In Vivo after Modified mRNA Vaccine Administration in Rhesus Macaques - ScienceDirect. Accessed March 2, 2022. https://www.sciencedirect.com/science/article/pii/S1525001617303659

74. Frontiers | Induction of Robust B Cell Responses after Influenza mRNA Vaccination Is Accompanied by Circulating Hemagglutinin-Specific ICOS+ PD-1+ CXCR3+ T Follicular Helper Cells | Immunology. Accessed March 2, 2022. https://www.frontiersin.org/articles/10.3389/fimmu.2017.01539/full

75. Moderna: mRNA-1273 – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/22/

76. Baden LR, El Sahly HM, Essink B, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. *N Engl J Med*. 2021;384(5):403-416. doi:10.1056/NEJMoa2035389

77. Richterman A, Scott J, Cevik M. Covid-19 vaccines, immunity, and boosters. *bmj*. 2021;375.

78. Chemaitelly H, Yassine HM, Benslimane FM, et al. mRNA-1273 COVID-19 vaccine effectiveness against the B. 1.1. 7 and B. 1.351 variants and severe COVID-19 disease in Qatar. *Nat Med*. 2021;27(9):1614-1621.

79. Pajon R, Doria-Rose NA, Shen X, et al. SARS-CoV-2 Omicron Variant Neutralization after mRNA-1273 Booster Vaccination. *N Engl J Med*. Published online 2022.

80. Takeda: TAK-919 (Moderna formulation) – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/79/

81. Takeda. *A Phase 1/2, Randomized, Observer-Blind, Placebo-Controlled Trial to Evaluate the Safety and Immunogenicity of TAK-919 by Intramuscular Injection in Healthy Japanese Male and Female Adults Aged 20 Years and Older (COVID-19)*. clinicaltrials.gov; 2021. Accessed December 16, 2021. https://clinicaltrials.gov/ct2/show/NCT04677660

82. Pfizer/BioNTech: BNT162b2 – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/6/

83. Tartof SY, Slezak JM, Fischer H, et al. Effectiveness of mRNA BNT162b2 COVID-19 vaccine up to 6 months in a large integrated health system in the USA: a retrospective cohort study. *The Lancet*. 2021;398(10309):1407-1416. doi:10.1016/S0140-6736(21)02183-8

84. Mahase E. Covid-19: Two vaccine doses are crucial for protection against delta, study finds. Published online 2021.

85. Nasreen S, Chung H, He S, et al. Effectiveness of COVID-19 vaccines against symptomatic SARS-CoV-2 infection and severe outcomes with variants of concern in Ontario. *Nat Microbiol*. Published online 2022:1-7.

86. Effectiveness of BNT162b2 Vaccine against Omicron Variant in South Africa | NEJM. Accessed March 2, 2022. https://www.nejm.org/doi/full/10.1056/NEJMc2119270

87. BNT162b2 mRNA Covid-19 Vaccine in a Nationwide Mass Vaccination Setting. Reply — Ben-Gurion University Research Portal. Accessed March 2, 2022. https://cris.bgu.ac.il/en/publications/bnt162b2-mrna-covid-19-vaccine-in-a-nationwide-mass-vaccination-s-2

88. Kow CS, Hasan SS. Real-world effectiveness of BNT162b2 mRNA vaccine: a meta-analysis of large observational studies. *Inflammopharmacology*. 2021;29(4):1075-1090.

89. Bruxvoort KJ, Sy LS, Qian L, et al. Real-world effectiveness of the mRNA-1273 vaccine against COVID-19: Interim results from a prospective observational cohort study. *Lancet Reg Health-Am*. Published online 2021:100134.

90. Zheng C, Shao W, Chen X, Zhang B, Wang G, Zhang W. Real-world effectiveness of COVID-19 vaccines: a literature review and meta-analysis. *Int J Infect Dis*. 2022;114:252-260.

91. Kadali RA, Janagama R, Peruru S, Malayala SV. Side effects of BNT162b2 mRNA COVID-19 vaccine: A randomized, cross-sectional study with detailed self-reported symptoms from healthcare workers. *Int J Infect Dis*. 2021;106:376-381.

92. Kadali RAK, Janagama R, Peruru S, et al. Non-life-threatening adverse effects with COVID-19 mRNA-1273 vaccine: A randomized, cross-sectional study on healthcare workers with detailed self-reported symptoms. *J Med Virol*. 2021;93(7):4420-4429.

93. Menni C, May A, Polidori L, et al. COVID-19 vaccine waning and effectiveness and side-effects of boosters: a prospective community study from the ZOE COVID Study. *Lancet Infect Dis*. 2022;22(7):1002-1010. doi:10.1016/S1473-3099(22)00146-3

94. Patalon T, Saciuk Y, Peretz A, et al. Waning effectiveness of the third dose of the BNT162b2 mRNA COVID-19 vaccine. *Nat Commun*. 2022;13(1):3203. doi:10.1038/s41467-022-30884-6

95. Goldberg Y, Mandel M, Bar-On YM, et al. Protection and Waning of Natural and Hybrid Immunity to SARS-CoV-2. *N Engl J Med*. 2022;386(23):2201-2212. doi:10.1056/NEJMoa2118946

96. Use of mRNA COVID-19 Vaccine After Reports of Myocarditis Among Vaccine Recipients: Update from the Advisory Committee on Immunization Practices — United States, June 2021 | MMWR. Accessed March 2, 2022. https://www.cdc.gov/mmwr/volumes/70/wr/mm7027e2.htm

97. Myocarditis With COVID-19 mRNA Vaccines | Circulation. Accessed March 2, 2022. https://www.ahajournals.org/doi/10.1161/CIRCULATIONAHA.121.056135

98. Mevorach D, Anis E, Cedar N, et al. Myocarditis after BNT162b2 mRNA vaccine against Covid-19 in Israel. *N Engl J Med*. 2021;385(23):2140-2149.

99. Montgomery J, Ryan M, Engler R, et al. Myocarditis following immunization with mRNA COVID-19 vaccines in members of the US military. *JAMA Cardiol*. 2021;6(10):1202-1206.

100. Barda N, Dagan N, Ben-Shlomo Y, et al. Safety of the BNT162b2 mRNA Covid-19 vaccine in a nationwide setting. *N Engl J Med*. Published online 2021.

101. Li H, Yu X, Liles C, et al. Autoimmune basis for postural tachycardia syndrome. *J Am Heart Assoc*. 2014;3(1):e000755.

102. Shiravi AA, Ardekani A, Sheikhbahaei E, Heshmat-Ghahdarijani K. Cardiovascular Complications of SARS-CoV-2 Vaccines: An Overview. *Cardiol Ther*. Published online 2021:1-9.

103. Reddy S, Reddy S, Arora M. A case of postural orthostatic tachycardia syndrome secondary to the messenger RNA COVID-19 vaccine. *Cureus*. 2021;13(5).

104. Chatterjee S, Ojha UK, Vardhan B, Tiwari A. Myocardial infarction after COVID-19 vaccination-casual or causal? *Diabetes Metab Syndr*. 2021;15(3):1055.

105. Boivin Z, Martin J. Untimely myocardial infarction or COVID-19 vaccine side effect. *Cureus*. 2021;13(3).

106. Tajstra M, Jaroszewicz J, Gąsior M. Acute coronary tree thrombosis after vaccination for COVID-19. *Cardiovasc Interv*. 2021;14(9):e103-e104.

107. Özdemir İH, Özlek B, Özen MB, Gündüz R, Bayturan Ö. Type 1 Kounis syndrome induced by inactivated SARS-COV-2 vaccine. *J Emerg Med*. 2021;61(4):e71-e76.

108. Meylan S, Livio F, Foerster M, et al. Stage III hypertension in patients after mRNA-based SARS-CoV-2 vaccination. *Hypertension*. 2021;77(6):e56-e57.

109. Zappa M, Verdecchia P, Spanevello A, Visca D, Angeli F. Blood pressure increase after Pfizer/BioNTech SARS-CoV-2 vaccine. *Eur J Intern Med*. 2021;90:111-113.

110. Jani C, Leavitt J, Al Omari O, et al. COVID-19 Vaccine–Associated Takotsubo Cardiomyopathy. *Am J Ther*. 2021;28(3):361-364.

111. Crane P, Wong C, Mehta N, Barlis P. Takotsubo (stress) cardiomyopathy after ChAdOx1 nCoV-19 vaccination. *BMJ Case Rep CP*. 2021;14(10):e246580.

112. Pepini T, Pulichino AM, Carsillo T, et al. Induction of an IFN-mediated antiviral response by a self-amplifying RNA vaccine: implications for vaccine design. *J Immunol*. 2017;198(10):4012-4024.

113. Pinschewer DD. Virally vectored vaccine delivery: medical needs, mechanisms, advantages and challenges. *Swiss Med Wkly*. 2017;147(3132).

114. de Bruyn G. Cofactors that may influence vaccine responses. *Curr Opin HIV AIDS*. 2010;5(5):404.

115. Thess A, Grund S, Mui BL, et al. Sequence-engineered mRNA without chemical nucleoside modifications enables an effective protein therapy in large animals. *Mol Ther*. 2015;23(9):1456-1464.

116. Andries O, Mc Cafferty S, De Smedt SC, Weiss R, Sanders NN, Kitada T. N1-methylpseudouridine-incorporated mRNA outperforms pseudouridine-incorporated mRNA by providing enhanced protein expression and reduced immunogenicity in mammalian cell lines and mice. *J Controlled Release*. 2015;217:337-344.

117. Kauffman KJ, Webber MJ, Anderson DG. Materials for non-viral intracellular delivery of messenger RNA therapeutics. *J Controlled Release*. 2016;240:227-234.

118. Sahin U, Karikó K, Türeci Ö. mRNA-based therapeutics—developing a new class of drugs. *Nat Rev Drug Discov*. 2014;13(10):759-780.

119. Li J, Ulitzky L, Silberstein E, Taylor DR, Viscidi R. Immunogenicity and protection efficacy of monomeric and trimeric recombinant SARS coronavirus spike protein subunit vaccine candidates. *Viral Immunol*. 2013;26(2):126-132.

120. He Y, Li J, Heck S, Lustigman S, Jiang S. Antigenic and immunogenic characterization of recombinant baculovirus-expressed severe acute respiratory syndrome coronavirus spike protein: implication for vaccine design. *J Virol*. 2006;80(12):5757-5767.

121. Kam YW, Kien F, Roberts A, et al. Antibodies against trimeric S glycoprotein protect hamsters against SARS-CoV challenge despite their capacity to mediate FcγRII-dependent entry into B cells in vitro. *Vaccine*. 2007;25(4):729-740.

122. He Y, Zhou Y, Liu S, et al. Receptor-binding domain of SARS-CoV spike protein induces highly potent neutralizing antibodies: implication for developing subunit vaccine. *Biochem Biophys Res Commun*. 2004;324(2):773-781.

123. Du L, Zhao G, Li L, et al. Antigenicity and immunogenicity of SARS-CoV S protein receptor-binding domain stably expressed in CHO cells. *Biochem Biophys Res Commun*. 2009;384(4):486-490.

124. Du L, Zhao G, He Y, et al. Receptor-binding domain of SARS-CoV spike protein induces long-term protective immunity in an animal model. *Vaccine*. 2007;25(15):2832-2838.

125. Du L, Zhao G, Kou Z, et al. Identification of a receptor-binding domain in the S protein of the novel human coronavirus Middle East respiratory syndrome coronavirus as an essential target for vaccine development. *J Virol*. 2013;87(17):9939-9942.

126. Dai L, Zheng T, Xu K, et al. A universal design of betacoronavirus vaccines against COVID-19, MERS, and SARS. *Cell*. 2020;182(3):722-733.

127. Lan J, Yao Y, Deng Y, et al. Recombinant receptor binding domain protein induces partial protective immunity in rhesus macaques against Middle East respiratory syndrome coronavirus challenge. *EBioMedicine*. 2015;2(10):1438-1446.

128. Ma C, Wang L, Tao X, et al. Searching for an ideal vaccine candidate among different MERS coronavirus receptor-binding fragments—the importance of immunofocusing in subunit vaccine design. *Vaccine*. 2014;32(46):6170-6176.

129. Lu G, Hu Y, Wang Q, et al. Molecular basis of binding between novel human coronavirus MERS-CoV and its receptor CD26. *Nature*. 2013;500(7461):227-231.

130. Adney DR, Wang L, van Doremalen N, et al. Efficacy of an Adjuvanted Middle East Respiratory Syndrome Coronavirus Spike Protein Vaccine in Dromedary Camels and Alpacas. *Viruses*. 2019;11(3):212. doi:10.3390/v11030212

131. Zhang S, Zhou P, Wang P, et al. Structural definition of a unique neutralization epitope on the receptor-binding domain of MERS-CoV spike glycoprotein. *Cell Rep*. 2018;24(2):441-452.

132. Tailoring Subunit Vaccine Immunity with Adjuvant Combinations and Delivery Routes Using the Middle East Respiratory Coronavirus (MERS-CoV) Receptor-Binding Domain as an Antigen. Accessed December 19, 2021. https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0112602

133. Cate TR, Couch RB, Kasel JA, Six HR. Clinical trials of monovalent influenza A/New Jersey/76 virus vaccines in adults: reactogenicity, antibody response, and antibody persistence. *J Infect Dis*. 1977;136(Supplement_3):S450-S455.

134. Brachman PS, Gold H, Plotkin SA, Fekety FR, Werrin M, Ingraham NR. Field evaluation of a human anthrax vaccine. *Am J Public Health Nations Health*. 1962;52(4):632-645.

135. Cox MM, Izikson R, Post P, Dunkle L. Safety, efficacy, and immunogenicity of Flublok in the prevention of seasonal influenza in adults. *Ther Adv Vaccines*. 2015;3(4):97-108.

136. Synthesis and assembly of hepatitis B virus surface antigen particles in yeast - PubMed. Accessed March 3, 2022. https://pubmed.ncbi.nlm.nih.gov/7045698/

137. Girard MP, Cherian T, Pervikov Y, Kieny MP. A review of vaccine research and development: human acute respiratory infections. *Vaccine*. 2005;23(50):5708-5724.

138. FBRI: EpiVacCorona – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/32/

139. Ryzhikov AB, Ryzhikov EA, Bogryantseva MP, 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(2):283-296. doi:10.15789/2220-7619-ASB-1699

140. Anhui Zhifei Longcom: ZF2001 – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/27/

141. Yang S, Li Y, Dai L, 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(8):1107-1119. doi:10.1016/S1473-3099(21)00127-4

142. Center for Genetic Engineering and Biotechnology (CIGB): CIGB-66 – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/67/

143. Evaluation of the safety and immunogenicity of the vaccine candidate "ABDALA" in children and adolescents | Registro Público Cubano de Ensayos Clínicos. Accessed December 19, 2021. https://rpcec.sld.cu/en/trials/RPCEC00000381-En

144. Instituto Finlay de Vacunas Cuba: Soberana 02 – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/52/

145. Safety and immunogenicity of anti-SARS CoV-2 vaccine SOBERANA 02 in homologous or heterologous scheme | medRxiv. Accessed December 19, 2021. https://www.medrxiv.org/content/10.1101/2021.11.14.21266309v1

146. Instituto Finlay de Vacunas Cuba: Soberana Plus – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/119/

147. Medigen: MVC-COV1901 – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/24/

148. Hsieh SM, Liu MC, Chen YH, et al. Safety and immunogenicity of CpG 1018 and aluminium hydroxide-adjuvanted SARS-CoV-2 S-2P protein vaccine MVC-COV1901: interim results of a large-scale, double-blind, randomised, placebo-controlled phase 2 trial in Taiwan. *Lancet Respir Med*. 2021;9(12):1396-1406. doi:10.1016/S2213-2600(21)00402-1

149. Razi Vaccine and Serum Research Institute: Razi Cov Pars – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/82/

150. IRCT | Phase II, Safety and Immunogenicity of RAZI SARS-CoV-2 recombinant Spike protein vaccine (RAZI Cov Pars) in adults aged 18-70 years; a Randomised, double blind, parallel 2 arms clinical trial. Accessed December 19, 2021. https://en.irct.ir/trial/55238

151. Serum Institute of India: COVOVAX (Novavax formulation) – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/123/

152. Novavax vaccine delivers 89% efficacy against COVID-19 in U.K.—but is less potent in South Africa | Science | AAAS. Accessed December 19, 2021.

https://www.science.org/content/article/novavax-vaccine-delivers-89-efficacy-against-covid-19-uk-less-potent-south-africa

153. Vaxine/CinnaGen Co.: COVAX-19 – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/8/

154. COVAX-19® Vaccine: Completely blocks virus transmission to non-immune individuals - ScienceDirect. Accessed December 19, 2021. https://www.sciencedirect.com/science/article/pii/S2772371221000048?via%3Dihub

155. Keech C, Albert G, Cho I, et al. Phase 1–2 trial of a SARS-CoV-2 recombinant spike protein nanoparticle vaccine. *N Engl J Med*. 2020;383(24):2320-2332.

156. Qian C, Liu X, Xu Q, et al. Recent progress on the versatility of virus-like particles. *Vaccines*. 2020;8(1):139.

157. Lokugamage KG, Yoshikawa-Iwata N, Ito N, et al. Chimeric coronavirus-like particles carrying severe acute respiratory syndrome coronavirus (SCoV) S protein protect mice against challenge with SCoV. *Vaccine*. 2008;26(6):797-808.

158. Tseng CT, Sbrana E, Iwata-Yoshikawa N, et al. Immunization with SARS coronavirus vaccines leads to pulmonary immunopathology on challenge with the SARS virus. *PloS One*. 2012;7(4):e35421.

159. Zeltins A. Construction and characterization of virus-like particles: a review. *Mol Biotechnol*. 2013;53(1):92-107.

160. Nooraei S, Bahrulolum H, Hoseini ZS, et al. Virus-like particles: Preparation, immunogenicity and their roles as nanovaccines and drug nanocarriers. *J Nanobiotechnology*. 2021;19(1):1-27.

161. Buonaguro L, Tagliamonte M, Tornesello ML, Buonaguro FM. Developments in virus-like particle-based vaccines for infectious diseases and cancer. *Expert Rev Vaccines*. 2011;10(11):1569-1583.

162. Bayer ME, Blumberg BS, Werner B. Particles associated with Australia antigen in the sera of patients with leukaemia, Down's Syndrome and hepatitis. *Nature*. 1968;218(5146):1057-1059.

163. Santi L, Huang Z, Mason H. Virus-like particles production in green plants. *Methods*. 2006;40(1):66-76.

164. Huang X, Wang X, Zhang J, Xia N, Zhao Q. Escherichia coli-derived virus-like particles in vaccine development. *Npj Vaccines*. 2017;2(1):1-9.

165. Akahata W, Yang ZY, Andersen H, et al. A virus-like particle vaccine for epidemic Chikungunya virus protects nonhuman primates against infection. *Nat Med*. 2010;16(3):334-338.

166. Hodgins B, Yam KK, Winter K, Pillet S, Landry N, Ward BJ. A Single Intramuscular Dose of a Plant-Made Virus-Like Particle Vaccine Elicits a Balanced Humoral and Cellular Response and Protects Young and Aged Mice from Influenza H1N1 Virus Challenge despite a Modest/Absent Humoral Response. *Clin Vaccine Immunol CVI*. 2017;24(12):e00273-17. doi:10.1128/CVI.00273-17

167. Madrid-Marina V, Torres-Poveda K, López-Toledo G, García-Carrancá A. Advantages and disadvantages of current prophylactic vaccines against HPV. *Arch Med Res*. 2009;40(6):471-477.

168. Fuenmayor J, Gòdia F, Cervera L. Production of virus-like particles for vaccines. *New Biotechnol*. 2017;39:174-180.

169. Bouard D, Alazard-Dany N, Cosset FL. Viral vectors: from virology to transgene expression. *Br J Pharmacol*. 2009;157(2):153-165.

170. Lee CS, Bishop ES, Zhang R, et al. Adenovirus-mediated gene delivery: Potential applications for gene and cell-based therapies in the new era of personalized medicine. *Genes Dis*. 2017;4(2):43-63. doi:10.1016/j.gendis.2017.04.001

171. Lauer KB, Borrow R, Blanchard TJ. Multivalent and multipathogen viral vector vaccines. *Clin Vaccine Immunol*. 2017;24(1):e00298-16.

172. SM Wold W, Toth K. Adenovirus vectors for gene therapy, vaccination and cancer gene therapy. *Curr Gene Ther*. 2013;13(6):421-433.

173. Zuniga A, Wang Z, Liniger M, et al. Attenuated measles virus as a vaccine vector. *Vaccine*. 2007;25(16):2974-2983.

174. Nato F, Boutonnier A, Rajerison M, et al. One-step immunochromatographic dipstick tests for rapid detection of Vibrio cholerae O1 and O139 in stool samples. *Clin Vaccine Immunol*. 2003;10(3):476-478.

175. Mühlebach MD. Vaccine platform recombinant measles virus. *Virus Genes*. 2017;53(5):733-740.

176. Letchworth GJ, Rodriguez LL, Del Cbarrera J. Vesicular stomatitis. *Vet J*. 1999;157(3):239-260.

177. Lawson ND, Stillman EA, Whitt MA, Rose JK. Recombinant vesicular stomatitis viruses from DNA. *Proc Natl Acad Sci*. 1995;92(10):4477-4481.

178. van den Pol AN, Davis JN. Highly attenuated recombinant vesicular stomatitis virus VSV-12' GFP displays immunogenic and oncolytic activity. *J Virol*. 2013;87(2):1019-1034.

179. Johnson JE, Nasar F, Coleman JW, et al. Neurovirulence properties of recombinant vesicular stomatitis virus vectors in non-human primates. *Virology*. 2007;360(1):36-49.

180. Tan WG, Jin HT, West EE, et al. Comparative analysis of simian immunodeficiency virus gag-specific effector and memory CD8+ T cells induced by different adenovirus vectors. *J Virol*. 2013;87(3):1359-1372.

181. Humphreys IR, Sebastian S. Novel viral vectors in infectious diseases. *Immunology*. 2018;153(1):1-9. doi:10.1111/imm.12829

182. Fausther-Bovendo H, Kobinger GP. Pre-existing immunity against Ad vectors: humoral, cellular, and innate response, what's important? *Hum Vaccines Immunother*. 2014;10(10):2875-2884. doi:10.4161/hv.29594

183. O'Hara GA, Duncan CJ, Ewer KJ, et al. Clinical assessment of a recombinant simian adenovirus ChAd63: a potent new vaccine vector. *J Infect Dis*. 2012;205(5):772-781.

184. Assessment of the Safety and Immunogenicity of 2 Novel Vaccine Platforms for HIV-1 Prevention: A Randomized Trial - PubMed. Accessed March 3, 2022. https://pubmed.ncbi.nlm.nih.gov/26833336/

185. Baldo A, Galanis E, Tangy F, Herman P. Biosafety considerations for attenuated measles virus vectors used in virotherapy and vaccination. *Hum Vaccines Immunother*. 2016;12(5):1102-1116.

186. Ramsauer K, Schwameis M, Firbas C, et al. Immunogenicity, safety, and tolerability of a recombinant measles-virus-based chikungunya vaccine: a randomised, double-blind, placebo-controlled, active-comparator, first-in-man trial. *Lancet Infect Dis*. 2015;15(5):519-527.

187. Green CA, Scarselli E, Voysey M, et al. Safety and immunogenicity of novel respiratory syncytial virus (RSV) vaccines based on the RSV viral proteins F, N and M2-1 encoded by simian adenovirus (PanAd3-RSV) and MVA (MVA-RSV); protocol for an open-label, dose-escalation, single-centre, phase 1 clinical trial in healthy adults. *BMJ Open*. 2015;5(10):e008748.

188. Meyer J, Harris SA, Satti I, et al. Comparing the safety and immunogenicity of a candidate TB vaccine MVA85A administered by intramuscular and intradermal delivery. *Vaccine*. 2013;31(7):1026-1033.

189. High titre neutralising antibodies to influenza after oral tablet immunisation: a phase 1, randomised, placebo-controlled trial - PubMed. Accessed March 4, 2022. https://pubmed.ncbi.nlm.nih.gov/26333337/

190. Peters W, Brandl JR, Lindbloom JD, et al. Oral administration of an adenovirus vector encoding both an avian influenza A hemagglutinin and a TLR3 ligand induces antigen specific granzyme B and IFN-γ T cell responses in humans. *Vaccine*. 2013;31(13):1752-1758.

191. Venkatraman N, Anagnostou N, Bliss C, et al. Safety and immunogenicity of heterologous prime-boost immunization with viral-vectored malaria vaccines adjuvanted with matrix-m™. *Vaccine*. 2017;35(45):6208-6217.

192. Robert-Guroff M. Replicating and non-replicating viral vectors for vaccine development. *Curr Opin Biotechnol*. 2007;18(6):546-556.

193. Ura T, Okuda K, Shimada M. Developments in viral vector-based vaccines. *Vaccines*. 2014;2(3):624-641.

194. Veit S, Jany S, Fux R, Sutter G, Volz A. CD8+ T Cells Responding to the Middle East Respiratory Syndrome Coronavirus Nucleocapsid Protein Delivered by Vaccinia Virus MVA in Mice. *Viruses*. 2018;10(12):E718. doi:10.3390/v10120718

195. Volz A, Kupke A, Song F, et al. Protective Efficacy of Recombinant Modified Vaccinia Virus Ankara Delivering Middle East Respiratory Syndrome Coronavirus Spike Glycoprotein. *J Virol*. 2015;89(16):8651-8656. doi:10.1128/JVI.00614-15

196. Sauter SL, Rahman A, Muralidhar G. Non-replicating viral vector-based AIDS vaccines: interplay between viral vectors and the immune system. *Curr HIV Res*. 2005;3(2):157-181.

197. Baxby D, Paoletti E. Potential use of non-replicating vectors as recombinant vaccines. *Vaccine*. 1992;10(1):8-9.

198. CanSino: Ad5-nCoV – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/2/

199. Safety, tolerability, and immunogenicity of an aerosolised adenovirus type-5 vector-based COVID-19 vaccine (Ad5-nCoV) in adults: preliminary report of an open-label and randomised phase 1 clinical trial - The Lancet Infectious Diseases. Accessed December 19, 2021. https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(21)00396-0/fulltext

200. Gamaleya: Sputnik V – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/12/

201. Jones I, Roy P. Sputnik V COVID-19 vaccine candidate appears safe and effective. *The Lancet*. 2021;397(10275):642-643. doi:10.1016/S0140-6736(21)00191-4

202. Gamaleya: Sputnik Light – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/126/

203. An open, non-randomised, phase 1/2 trial on the safety, tolerability, and immunogenicity of single-dose vaccine "Sputnik Light" for prevention of coronavirus infection in healthy adults - The Lancet Regional Health – Europe. Accessed December 19, 2021. https://www.thelancet.com/journals/lanepe/article/PIIS2666-7762(21)00227-1/fulltext

204. Janssen (Johnson & Johnson): Ad26.COV2.S – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/1/

205. Safety and Efficacy of Single-Dose Ad26.COV2.S Vaccine against Covid-19 | NEJM. Accessed December 19, 2021. https://www.nejm.org/doi/full/10.1056/NEJMoa2101544

206. 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 - The Lancet. Accessed December 19, 2021. https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)32661-1/fulltext

207. WHO 2019 NCoV Vaccines SAGE Recommendation AZD1222 2021.3 Eng | PDF | Pregnancy | Vaccines. Scribd. Accessed December 19, 2021. https://www.scribd.com/document/531662920/WHO-2019-NCoV-Vaccines-SAGE-Recommendation-AZD1222-2021-3-Eng

208. Oxford/AstraZeneca: AZD1222 – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/4/

209. Serum Institute of India: Covishield (Oxford/AstraZeneca formulation) – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/48/

210. Madhi SA, Baillie V, Cutland CL, et al. Wits-VIDA COVID Group.(2021). *Effic ChAdOx1 NCoV-19 Covid-19 Vaccine B*. 1:1885-1898.

211. Andrews N, Stowe J, Kirsebom F, et al. Effectiveness of COVID-19 vaccines against the Omicron (B. 1.1. 529) variant of concern. *MedRxiv*. Published online 2021.

212. Klugar M, Riad A, Mekhemar M, et al. Side effects of mRNA-based and viral vector-based COVID-19 vaccines among German healthcare workers. *Biology*. 2021;10(8):752.

213. Voysey M, Clemens SAC, Madhi SA, 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. *The Lancet*. 2021;397(10269):99-111.

214. Woo EJ, Mba-Jonas A, Dimova RB, Alimchandani M, Zinderman CE, Nair N. Association of receipt of the Ad26. COV2. S COVID-19 vaccine with presumptive Guillain-Barré syndrome, February-July 2021. *JAMA*. 2021;326(16):1606-1613.

215. Sadoff J, Davis K, Douoguih M. Thrombotic thrombocytopenia after Ad26. COV2. S vaccination—response from the manufacturer. *N Engl J Med*. 2021;384(20):1965-1966.

216. Burn E, Li X, Kostka K, et al. Background rates of five thrombosis with thrombocytopenia syndromes of special interest for COVID-19 vaccine safety surveillance: incidence between 2017 and 2019 and patient profiles from 20.6 million people in six European countries. *medRxiv*. Published online 2021.

217. Gupta A, Sardar P, Cash ME, Milani RV, Lavie CJ. Covid-19 vaccine-induced thrombosis and thrombocytopenia-a commentary on an important and practical clinical dilemma. *Prog Cardiovasc Dis*. Published online 2021.

218. Commissioner O of the. Joint CDC and FDA Statement on Johnson & Johnson COVID-19 Vaccine. FDA. Published April 13, 2021. Accessed March 4, 2022. https://www.fda.gov/news-events/press-announcements/joint-cdc-and-fda-statement-johnson-johnson-covid-19-vaccine

219. Pottegård A, Lund LC, Karlstad Ø, et al. Arterial events, venous thromboembolism, thrombocytopenia, and bleeding after vaccination with Oxford-AstraZeneca ChAdOx1-S in Denmark and Norway: population based cohort study. *bmj*. 2021;373.

220. Choo SZL, Teo SP. Gam-COVID-Vac (Sputnik V): a heterologous adenoviral vector-based COVID-19 vaccine. *Aging Pathobiol Ther*. 2021;3(2):43-45.

221. Baldo A, van den Akker E, E Bergmans H, Lim F, Pauwels K. General considerations on the biosafety of virus-derived vectors used in gene therapy and vaccination. *Curr Gene Ther*. 2013;13(6):385-394.

222. Condit RC, Williamson AL, Sheets R, et al. Unique safety issues associated with virus-vectored vaccines: Potential for and theoretical consequences of recombination with wild type virus strains. *Vaccine*. 2016;34(51):6610-6616.

223. Klug B, Robertson JS, Condit RC, et al. Adventitious agents and live viral vectored vaccines: considerations for archiving samples of biological materials for retrospective analysis. *Vaccine*. 2016;34(51):6617-6625.

224. Vax Report - Understanding Pre-existing Immunity. Accessed March 4, 2022. https://www.vaxreport.org/vax-3-2-february-2005/801-understanding-pre-existing-immunity

225. Sette A, Crotty S. Pre-existing immunity to SARS-CoV-2: the knowns and unknowns. *Nat Rev Immunol*. 2020;20(8):457-458. doi:10.1038/s41577-020-0389-z

226. Cromer D, Reynaldi A, Steain M, Triccas JA, Davenport MP, Khoury DS. Relating in vitro neutralisation level and protection in the CVnCoV (CUREVAC) trial. *medRxiv*. Published online 2021.

227. Classification of Vaccines | SpringerLink. Accessed August 27, 2021. https://link.springer.com/chapter/10.1007/978-1-4939-1417-3_2

228. Bolles M, Deming D, Long K, et al. A double-inactivated severe acute respiratory syndrome coronavirus vaccine provides incomplete protection in mice and induces increased eosinophilic proinflammatory pulmonary response upon challenge. *J Virol*. 2011;85(23):12201-12215. doi:10.1128/JVI.06048-11

229. Reperant LA, Osterhaus ADME. AIDS, Avian flu, SARS, MERS, Ebola, Zika… what next? *Vaccine*. 2017;35(35 Pt A):4470-4474. doi:10.1016/j.vaccine.2017.04.082

230. Xiong S, Wang YF, Zhang MY, et al. Immunogenicity of SARS inactivated vaccine in BALB/c mice. *Immunol Lett*. 2004;95(2):139-143. doi:10.1016/j.imlet.2004.06.014

231. Takasuka N, Fujii H, Takahashi Y, et al. A subcutaneously injected UV-inactivated SARS coronavirus vaccine elicits systemic humoral immunity in mice. *Int Immunol*. 2004;16(10):1423-1430. doi:10.1093/intimm/dxh143

232. Qin E, Shi H, Tang L, et al. Immunogenicity and protective efficacy in monkeys of purified inactivated Vero-cell SARS vaccine. *Vaccine*. 2006;24(7):1028-1034. doi:10.1016/j.vaccine.2005.06.038

233. Deng Y, Lan J, Bao L, et al. Enhanced protection in mice induced by immunization with inactivated whole viruses compare to spike protein of middle east respiratory syndrome coronavirus. *Emerg Microbes Infect*. 2018;7(1):60. doi:10.1038/s41426-018-0056-7

234. Yasui F, Kai C, Kitabatake M, et al. Prior immunization with severe acute respiratory syndrome (SARS)-associated coronavirus (SARS-CoV) nucleocapsid protein causes severe pneumonia in mice infected with SARS-CoV. *J Immunol Baltim Md 1950*. 2008;181(9):6337-6348. doi:10.4049/jimmunol.181.9.6337

235. Petrovsky N, Aguilar JC. Vaccine adjuvants: current state and future trends. *Immunol Cell Biol*. 2004;82(5):488-496.

236. Plotkin SA, Plotkin SL. The development of vaccines: how the past led to the future. *Nat Rev Microbiol*. 2011;9(12):889-893.

237. Witter RL. Principles of vaccination. In: *Marek's Disease*. Springer; 1985:203-250.

238. Vetter V, Denizer G, Friedland LR, Krishnan J, Shapiro M. Understanding modern-day vaccines: what you need to know. *Ann Med*. 2018;50(2):110-120.

239. Slifka MK, Amanna I. How advances in immunology provide insight into improving vaccine efficacy. *Vaccine*. 2014;32(25):2948-2957.

240. Pollard AJ, Bijker EM. A guide to vaccinology: from basic principles to new developments. *Nat Rev Immunol*. 2021;21(2):83-100.

241. Ellis RW. New technologies for making vaccines. *Vaccine*. 1999;17(13-14):1596-1604.

242. Guzman CA, Borsutzky S, Griot-Wenk M, et al. Vaccines against typhoid fever. *Vaccine*. 2006;24(18):3804-3811. doi:10.1016/j.vaccine.2005.07.111

243. Ryan ET, Calderwood SB. Cholera vaccines. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2000;31(2):561-565. doi:10.1086/313951

244. Krugman S. Hepatitis virus vaccines: present status. *Yale J Biol Med*. 1982;55(3-4):375-381.

245. Jefferson T, Demicheli V, Pratt M. Vaccines for preventing plague. *Cochrane Database Syst Rev*. 2000;(2):CD000976. doi:10.1002/14651858.CD000976

246. Hicks DJ, Fooks AR, Johnson N. Developments in rabies vaccines. *Clin Exp Immunol*. 2012;169(3):199-204. doi:10.1111/j.1365-2249.2012.04592.x

247. Demicheli V, Jefferson T, Di Pietrantonj C, et al. Vaccines for preventing influenza in the elderly. *Cochrane Database Syst Rev*. 2018;2:CD004876. doi:10.1002/14651858.CD004876.pub4

248. Jefferson T, Rivetti A, Di Pietrantonj C, Demicheli V. Vaccines for preventing influenza in healthy children. *Cochrane Database Syst Rev*. 2018;2:CD004879. doi:10.1002/14651858.CD004879.pub5

249. Ehrenfeld E, Modlin J, Chumakov K. Future of polio vaccines. *Expert Rev Vaccines*. 2009;8(7):899-905. doi:10.1586/erv.09.49

250. Vashishtha VM, Kamath S. A Brief History of Vaccines Against Polio. *Indian Pediatr*. 2016;53 Suppl 1:S20-S27.

251. Bharat Biotech: Covaxin – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/9/

252. Ella R, Reddy S, Blackwelder W, 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. *The Lancet*. 2021;398(10317):2173-2184. doi:10.1016/S0140-6736(21)02000-6

253. Effect of 2 Inactivated SARS-CoV-2 Vaccines on Symptomatic COVID-19 Infection in Adults: A Randomized Clinical Trial | Global Health | JAMA | JAMA Network. Accessed December 19, 2021. https://jamanetwork.com/journals/jama/fullarticle/2780562

254. Sinopharm (Beijing): BBIBP-CorV (Vero Cells) – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/5/

255. Sinopharm (Wuhan): Inactivated (Vero Cells) – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/16/

256. Sinovac: CoronaVac – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/7/

257. Interim report: Safety and immunogenicity of an inactivated vaccine against SARS-CoV-2 in healthy chilean adults in a phase 3 clinical trial | medRxiv. Accessed December 19, 2021. https://www.medrxiv.org/content/10.1101/2021.03.31.21254494v1

258. Han B, Song Y, Li C, et al. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy children and adolescents: a double-blind, randomised, controlled, phase 1/2 clinical trial. *Lancet Infect Dis*. 2021;21(12):1645-1653. doi:10.1016/S1473-3099(21)00319-4

259. Chumakov Center: KoviVac – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/100/

260. Kozlovskaya LI, Piniaeva AN, Ignatyev GM, et al. Long-term humoral immunogenicity, safety and protective efficacy of inactivated vaccine against COVID-19 (CoviVac) in preclinical studies. *Emerg Microbes Infect*. 2021;10(1):1790-1806. doi:10.1080/22221751.2021.1971569

261. Kazakhstan RIBSP: QazVac – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/30/

262. Zakarya K, Kutumbetov L, Orynbayev M, et al. Safety and immunogenicity of a QazCovid-in® inactivated whole-virion vaccine against COVID-19 in healthy adults: A single-centre, randomised, single-blind, placebo-controlled phase 1 and an open-label phase 2 clinical trials with a 6 months follow-up in Kazakhstan. *EClinicalMedicine*. 2021;39:101078. doi:10.1016/j.eclinm.2021.101078

263. Minhai Biotechnology Co: SARS-CoV-2 Vaccine (Vero Cells) – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/47/

264. Pan H, Liu J, Huang B, et al. *Immunogenicity and Safety of a SARS-CoV-2 Inactivated Vaccine (KCONVAC) in Healthy Adults: Two Randomized, Double-Blind, and Placebo-Controlled Phase 1/2 Clinical Trials*.; 2021:2021.04.07.21253850. doi:10.1101/2021.04.07.21253850

265. Organization of Defensive Innovation and Research: FAKHRAVAC (MIVAC) – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/97/

266. IRCT | Comparison of the safety, efficacy and immunogenicity of Fakhravac and Sinopharm SARS-CoV-2 vaccines, in adults aged 18 and over; a phase III randomised, non-inferiority clinical trial. Accessed December 19, 2021. https://en.irct.ir/trial/57980

267. Shifa Pharmed Industrial Co: COVID-19 Inactivated Vaccine – COVID19 Vaccine Tracker. Accessed December 19, 2021. https://covid19.trackvaccines.org/vaccines/83/

268. Abdoli A, Aalizadeh R, Aminianfar H, et al. *Safety and Potency of COVIran Barekat Inactivated Vaccine Candidate for SARS-CoV-2: A Preclinical Study*.; 2021:2021.06.10.447951. doi:10.1101/2021.06.10.447951

269. Arora P, Sardana K, Mathachan SR, Malhotra P. Herpes zoster after inactivated COVID-19 vaccine: a cutaneous adverse effect of the vaccine. *J Cosmet Dermatol*. Published online 2021.

270. Cebeci F, Kartal İ. Petechial skin rash associated with CoronaVac vaccination: first cutaneous side effect report before phase 3 results. *Eur J Hosp Pharm*. Published online 2021.

271. Clem AS. Fundamentals of vaccine immunology. *J Glob Infect Dis*. 2011;3(1):73.

272. Minor PD. Live attenuated vaccines: Historical successes and current challenges. *Virology*. 2015;479-480:379-392. doi:10.1016/j.virol.2015.03.032

273. Jordan I, Sandig V. Matrix and backstage: cellular substrates for viral vaccines. *Viruses*. 2014;6(4):1672-1700.

274. Vaccine Analysis: Strategies, Principles, and Control | SpringerLink. Accessed March 4, 2022. https://link.springer.com/book/10.1007/978-3-662-45024-6

275. Hanley KA. The double-edged sword: How evolution can make or break a live-attenuated virus vaccine. *Evol Educ Outreach*. 2011;4(4):635-643.

276. Gasparini R, Amicizia D, Lai PL, Panatto D. Live attenuated influenza vaccine–a review. *J Prev Med Hyg*. 2011;52(3):95-101.

277. Plotkin SA, Orenstein W, Offit PA. *Vaccines E-Book*. Elsevier Health Sciences; 2012.

278. Herzog C. Influence of parenteral administration routes and additional factors on vaccine safety and immunogenicity: a review of recent literature. *Expert Rev Vaccines*. 2014;13(3):399-415.

279. Tai W, Wang Y, Fett CA, et al. Recombinant Receptor-Binding Domains of Multiple Middle East Respiratory Syndrome Coronaviruses (MERS-CoVs) Induce Cross-Neutralizing Antibodies against Divergent Human and Camel MERS-CoVs and Antibody Escape Mutants. *J Virol*. 2017;91(1):e01651-16. doi:10.1128/JVI.01651-16

280. Fett C, DeDiego ML, Regla-Nava JA, Enjuanes L, Perlman S. Complete protection against severe acute respiratory syndrome coronavirus-mediated lethal respiratory disease in aged mice by immunization with a mouse-adapted virus lacking E protein. *J Virol*. 2013;87(12):6551-6559. doi:10.1128/JVI.00087-13

281. Lamirande EW, DeDiego ML, Roberts A, et al. A live attenuated severe acute respiratory syndrome coronavirus is immunogenic and efficacious in golden Syrian hamsters. *J Virol*. 2008;82(15):7721-7724. doi:10.1128/JVI.00304-08

282. Vignuzzi M, Wendt E, Andino R. Engineering attenuated virus vaccines by controlling replication fidelity. *Nat Med*. 2008;14(2):154-161. doi:10.1038/nm1726

283. Yadav DK, Yadav N, Khurana SMP. Vaccines: present status and applications. In: *Animal Biotechnology*. Elsevier; 2020:523-542.

284. Strizova Z, Smetanova J, Bartunkova J, Milota T. Principles and Challenges in anti-COVID-19 Vaccine Development. *Int Arch Allergy Immunol*. 2021;182(4):339-349. doi:10.1159/000514225

285. Peng S, Cao F, Xia Y, et al. Particulate Alum via Pickering Emulsion for an Enhanced COVID-19 Vaccine Adjuvant. *Adv Mater Deerfield Beach Fla*. 2020;32(40):e2004210. doi:10.1002/adma.202004210

286. Liu Z, Zhou J, Xu W, et al. A novel STING agonist-adjuvanted pan-sarbecovirus vaccine elicits potent and durable neutralizing antibody and T cell responses in mice, rabbits and NHPs. *Cell Res*. 2022;32(3):269-287. doi:10.1038/s41422-022-00612-2

287. Van Herck S, Feng B, Tang L. Delivery of STING agonists for adjuvanting subunit vaccines. *Adv Drug Deliv Rev*. 2021;179:114020. doi:10.1016/j.addr.2021.114020

288. Zhang R, Wang C, Guan Y, et al. Manganese salts function as potent adjuvants. *Cell Mol Immunol*. 2021;18(5):1222-1234. doi:10.1038/s41423-021-00669-w

289. Lv M, Chen M, Zhang R, et al. Manganese is critical for antitumor immune responses via cGAS-STING and improves the efficacy of clinical immunotherapy. *Cell Res*. 2020;30(11):966-979. doi:10.1038/s41422-020-00395-4

290. Chappell KJ, Mordant FL, Li Z, et al. Safety and immunogenicity of an MF59-adjuvanted spike glycoprotein-clamp vaccine for SARS-CoV-2: a randomised, double-blind, placebo-controlled, phase 1 trial. *Lancet Infect Dis*. 2021;21(10):1383-1394. doi:10.1016/S1473-3099(21)00200-0

291. Francica JR, Flynn BJ, Foulds KE, et al. Protective antibodies elicited by SARS-CoV-2 spike protein vaccination are boosted in the lung after challenge in nonhuman primates. *Sci Transl Med*. 2021;13(607):eabi4547. doi:10.1126/scitranslmed.abi4547

292. Takeuchi O, Akira S. Pattern recognition receptors and inflammation. *Cell*. 2010;140(6):805-820. doi:10.1016/j.cell.2010.01.022

293. Kurup D, Malherbe DC, Wirblich C, et al. Inactivated rabies virus vectored SARS-CoV-2 vaccine prevents disease in a Syrian hamster model. *PLoS Pathog*. 2021;17(3):e1009383. doi:10.1371/journal.ppat.1009383

294. Jeong SK, Heo YK, Jeong JH, et al. COVID-19 Subunit Vaccine with a Combination of TLR1/2 and TLR3 Agonists Induces Robust and Protective Immunity. *Vaccines*. 2021;9(9):957. doi:10.3390/vaccines9090957

295. Zhou SH, Zhang RY, Zhang HW, et al. RBD conjugate vaccine with a built-in TLR1/2 agonist is highly immunogenic against SARS-CoV-2 and variants of concern. *Chem Commun Camb Engl*. 2022;58(13):2120-2123. doi:10.1039/d1cc06520c

296. Lei H, Alu A, Yang J, et al. Cationic nanocarriers as potent adjuvants for recombinant S-RBD vaccine of SARS-CoV-2. *Signal Transduct Target Ther*. 2020;5(1):291. doi:10.1038/s41392-020-00434-x

297. Formica N, Mallory R, Albert G, et al. Different dose regimens of a SARS-CoV-2 recombinant spike protein vaccine (NVX-CoV2373) in younger and older adults: A phase 2 randomized placebo-controlled trial. *PLoS Med*. 2021;18(10):e1003769. doi:10.1371/journal.pmed.1003769

298. Li L, Honda-Okubo Y, Huang Y, et al. Immunisation of ferrets and mice with recombinant SARS-CoV-2 spike protein formulated with Advax-SM adjuvant protects against COVID-19 infection. *Vaccine*. 2021;39(40):5940-5953. doi:10.1016/j.vaccine.2021.07.087

299. Lapuente D, Fuchs J, Willar J, et al. Protective mucosal immunity against SARS-CoV-2 after heterologous systemic prime-mucosal boost immunization. *Nat Commun*. 2021;12(1):1-14.

300. Analysis of paraprotein transport into the saliva by using anti-idiotype antibodies - PubMed. Accessed March 4, 2022. https://pubmed.ncbi.nlm.nih.gov/3794339/

301. Nahass GR, Salomon-Shulman RE, Blacker G, et al. Intramuscular SARS-CoV-2 vaccines elicit varying degrees of plasma and salivary antibody responses as compared to natural infection. *medRxiv*. Published online 2021.

302. Ketas TJ, Chaturbhuj D, Portillo VMC, et al. Antibody responses to SARS-CoV-2 mRNA vaccines are detectable in saliva. *Pathog Immun*. 2021;6(1):116.

303. Russell MW, Moldoveanu Z, Ogra PL, Mestecky J. Mucosal immunity in COVID-19: a neglected but critical aspect of SARS-CoV-2 infection. *Front Immunol*. Published online 2020:3221.

304. Pabst R. Mucosal vaccination by the intranasal route. Nose-associated lymphoid tissue (NALT)—Structure, function and species differences. *Vaccine*. 2015;33(36):4406-4413.

305. Increased pregnancy problems with COVID-19–meta-analysi... - Google Scholar. Accessed March 2, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Increased+pregnancy+problems+with+COVID-19%E2%80%93meta-analysis+and+letter+to+editor%E2%80%93April+2021&btnG=

306. COVID-19 Vaccines for Specific Groups of People | CDC. Accessed March 3, 2022. https://www.cdc.gov/coronavirus/2019-ncov/vaccines/recommendations/specific-groups.html

307. Previous psychopathology predicted severe COVID-19... - Google Scholar. Accessed March 2, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Previous+psychopathology+predicted+severe+COVID-19+concern%2C+anxiety%2C+and+PTSD+symptoms+in+pregnant+women+during+%E2%80%9Clockdown%E2%80%9D+in+Italy&btnG=

308. COVID-19 vaccination in pregnancy, paediatrics, immunocom... - Google Scholar. Accessed March 2, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=COVID-19+vaccination+in+pregnancy%2C+paediatrics%2C+immunocompromised+patients%2C+and+persons+with+history+of+allergy+or+prior+SARS-CoV-2+infection%3A+overview+%E2%80%A6&btnG=

309. Update: Characteristics of Symptomatic Women of Reproductive Age with Laboratory-Confirmed SARS-CoV-2 Infection by Pregnancy Status — United States, January 22–October 3, 2020 | MMWR. Accessed March 2, 2022. https://www.cdc.gov/mmwr/volumes/69/wr/mm6944e3.htm?s_cid=mm6944e3_w

310. Pregnancy During Covid-19 — Prevent Child Abuse Vermont. Accessed March 2, 2022. https://www.pcavt.org/parents-corner-blog/2020/6/9/pregnancy-during-covid-19?gclid=CjwKCAiAyPyQBhB6EiwAFUuakp6jW6Xc0mHI3yxQaWDDw_Zn9QT4yi2dKuoDHBcrz-vzcGRvVFvkUhoCjtUQAvD_BwE

311. Effectiveness of the BNT162b2 mRNA COVID-19 vaccine... - Google Scholar. Accessed March 2, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Effectiveness+of+the+BNT162b2+mRNA+COVID-19+vaccine+in+pregnancy&btnG=

312. Association between BNT162b2 vaccination and incidence... - Google Scholar. Accessed March 2, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Association+between+BNT162b2+vaccination+and+incidence+of+SARS-CoV-2+infection+in+pregnant+women&btnG=

313. Maternal COVID-19 vaccine antibody response and passage... - Google Scholar. Accessed March 2, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Maternal+COVID-19+vaccine+antibody+response+and+passage+into+cord+blood&btnG=

314. SARS-CoV-2–specific antibodies in breast milk after... - Google Scholar. Accessed March 2, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=SARS-CoV-2%E2%80%93specific+antibodies+in+breast+milk+after+COVID-19+vaccination+of+breastfeeding+women&btnG=

315. Update on WHO Interim recommendations on COVID-19 vaccination of pregnant and lactating women. Accessed March 2, 2022. https://www.who.int/publications/m/item/update-on-who-interim-recommendations-on-covid-19-vaccination-of-pregnant-and-lactating-women

316. COVID-19 vaccines, pregnancy and breastfeeding. Accessed March 2, 2022. https://www.rcog.org.uk/en/guidelines-research-services/coronavirus-covid-19-pregnancy-and-womens-health/covid-19-vaccines-and-pregnancy/covid-19-vaccines-pregnancy-and-breastfeeding/

317. Suppression of a SARS-CoV-2 outbreak in the Italian... - Google Scholar. Accessed March 3, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Suppression+of+a+SARS-CoV-2+outbreak+in+the+Italian+municipality+of+Vo%27&btnG=

318. Coronavirus disease-19: the first 7,755 cases in... - Google Scholar. Accessed March 3, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Coronavirus+disease-19%3A+the+first+7%2C755+cases+in+the+Republic+of+Korea&btnG=

319. Spread of SARS-CoV-2 in the Icelandic population - Google Scholar. Accessed March 3, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Spread+of+SARS-CoV-2+in+the+Icelandic+population&btnG=

320. Epidemiological characteristics of 2143 pediatric... - Google Scholar. Accessed March 3, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Epidemiological+characteristics+of+2143+pediatric+patients+with+2019+coronavirus+disease+in+China&btnG=

321. Clinical characteristics of novel coronavirus disease... - Google Scholar. Accessed March 3, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Clinical+characteristics+of+novel+coronavirus+disease+2019+%28COVID-19%29+in+newborns%2C+infants+and+children&btnG=

322. First results of the SARS-CoV-2 seroprevalence survey. Accessed March 3, 2022. https://www.istat.it/it/archivio/246156

323. stayhome to contain Covid-19: Neuro-SIR–Neurodynamical... - Google Scholar. Accessed March 3, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=stayhome+to+contain+Covid-19%3A+Neuro-SIR%E2%80%93Neurodynamical+epidemic+modeling+of+infection+patterns+in+social+networks&btnG=

324. Multisystem inflammatory syndrome in children and adolescents temporally related to COVID-19. Accessed March 3, 2022. https://www.who.int/news-room/commentaries/detail/multisystem-inflammatory-syndrome-in-children-and-adolescents-with-covid-19

325. Comirnaty and Pfizer-BioNTech COVID-19 Vaccine | FDA. Accessed March 3, 2022. https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/comirnaty-and-pfizer-biontech-covid-19-vaccine#additional

326. COVID-19 in Immunocompromised Hosts: What We Know So Far - Google Scholar. Accessed March 3, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=COVID-19+in+Immunocompromised+Hosts%3A+What+We+Know+So+Far&btnG=

327. Association of clinical factors and recent anticancer... - Google Scholar. Accessed March 3, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Association+of+clinical+factors+and+recent+anticancer+therapy+with+COVID-19+severity+among+patients+with+cancer%3A+a+report+from+the+COVID-19+and+Cancer+%E2%80%A6&btnG=

328. Prevalence and clinical outcomes of COVID-19 in patients... - Google Scholar. Accessed March 3, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Prevalence+and+clinical+outcomes+of+COVID-19+in+patients+with+autoimmune+diseases%3A+a+systematic+review+and+meta-analysis&btnG=

329. Biomedicines | Free Full-Text | Anti-COVID-19 Vaccination in Patients with Autoimmune-Autoinflammatory Disorders and Primary/Secondary Immunodeficiencies: The Position of the Task Force on Behalf of the Italian Immunological Societies. Accessed March 3, 2022. https://www.mdpi.com/2227-9059/9/9/1163

330. Immunogenicity of a single dose of SARS-CoV-2 messenger... - Google Scholar. Accessed March 3, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Immunogenicity+of+a+single+dose+of+SARS-CoV-2+messenger+RNA+vaccine+in+solid+organ+transplant+recipients&btnG=

331. Spike-antibody responses following first and second... - Google Scholar. Accessed March 3, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Spike-antibody+responses+following+first+and+second+doses+of+ChAdOx1+and+BNT162b2+vaccines+by+age%2C+gender%2C+and+clinical+factors-a+prospective+%E2%80%A6&btnG=

332. Impaired humoral immunity to SARS-CoV-2 BNT162b2... - Google Scholar. Accessed March 3, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Impaired+humoral+immunity+to+SARS-CoV-2+BNT162b2+vaccine+in+kidney+transplant+recipients+and+dialysis+patients&btnG=

333. Immunogenicity of BNT162b2 mRNA COVID-19 vaccine... - Google Scholar. Accessed March 3, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Immunogenicity+of+BNT162b2

+mRNA+COVID-19+vaccine+and+SARS-CoV-2+infection+in+lung+transplant+recipients&btnG=

334. Herpes zoster following BNT162b2 mRNA Covid-19 vaccinatio... - Google Scholar. Accessed March 3, 2022. https://scholar.google.com/scholar?hl=en&as_sdt=0%2C5&q=Herpes+zoster+following+BNT162b2+mRNA+Covid-19+vaccination+in+patients+with+autoimmune+inflammatory+rheumatic+diseases%3A+a+case+series&btnG=

335. Pfizer-BioNTech and Oxford AstraZeneca COVID-19 vaccine effectiveness and immune response among individuals in clinical risk groups — Nuffield Department of Primary Care Health Sciences, University of Oxford. Accessed March 3, 2022. https://www.phc.ox.ac.uk/publications/1230305

336. ASCIA HP Position Statement COVID-19 Vaccination - Australasian Society of Clinical Immunology and Allergy (ASCIA). Accessed March 3, 2022. https://www.allergy.org.au/hp/papers/ascia-hp-position-statement-covid-19-vaccination

337. Haberman RH, Herati R, Simon D, et al. Methotrexate hampers immunogenicity to BNT162b2 mRNA COVID-19 vaccine in immune-mediated inflammatory disease. *Ann Rheum Dis*. 2021;80(10):1339-1344. doi:10.1136/annrheumdis-2021-220597

338. Alexander JL, Kennedy NA, Ibraheim H, et al. COVID-19 vaccine-induced antibody responses in immunosuppressed patients with inflammatory bowel disease (VIP): a multicentre, prospective, case-control study. *Lancet Gastroenterol Hepatol*. 2022;7(4):342-352. doi:10.1016/S2468-1253(22)00005-X

339. Smetanova J, Strizova Z, Sediva A, Milota T, Horvath R. Humoral and cellular immune responses to mRNA COVID-19 vaccines in patients with axial spondyloarthritis treated with adalimumab or secukinumab. *Lancet Rheumatol*. 2022;4(3):e163-e166. doi:10.1016/S2665-9913(21)00393-3

340. Yamashita M, Inoue K, Okano T, Morio T. Inborn errors of immunity-recent advances in research on the pathogenesis. *Inflamm Regen*. 2021;41(1):9. doi:10.1186/s41232-021-00159-6

341. Kumar S, Basu M, Ghosh MK. Chaperone-assisted E3 ligase CHIP: A double agent in cancer. *Genes Dis*. 2021;9(6). doi:10.1016/j.gendis.2021.08.003

342. Kumar S, Chatterjee M, Ghosh P, Ganguly KK, Basu M, Ghosh MK. Targeting PD-1/PD-L1 in cancer immunotherapy: an effective strategy for treatment of triple-negative breast cancer (TNBC) patients. *Genes Dis*. Published online August 24, 2022. doi:10.1016/j.gendis.2022.07.024

343. Abraham RS, Marshall JM, Kuehn HS, et al. Severe SARS-CoV-2 disease in the context of a NF-κB2 loss-of-function pathogenic variant. *J Allergy Clin Immunol*. 2021;147(2):532-544.e1. doi:10.1016/j.jaci.2020.09.020

344. Meyts I, Bucciol G, Quinti I, et al. Coronavirus disease 2019 in patients with inborn errors of immunity: An international study. *J Allergy Clin Immunol*. 2021;147(2):520-531. doi:10.1016/j.jaci.2020.09.010

345. VanBlargan LA, Errico JM, Halfmann PJ, et al. An infectious SARS-CoV-2 B.1.1.529 Omicron virus escapes neutralization by therapeutic monoclonal antibodies. *Nat Med*. 2022;28(3):490-495. doi:10.1038/s41591-021-01678-y

346. Karakoc Aydiner E, Bilgic Eltan S, Babayeva R, et al. Adverse COVID-19 outcomes in immune deficiencies: Inequality exists between subclasses. *Allergy*. 2022;77(1):282-295. doi:10.1111/all.15025

347. Delavari S, Abolhassani H, Abolnezhadian F, et al. Impact of SARS-CoV-2 Pandemic on Patients with Primary Immunodeficiency. *J Clin Immunol*. 2021;41(2):345-355. doi:10.1007/s10875-020-00928-x

348. Kinoshita H, Durkee-Shock J, Jensen-Wachspress M, et al. Robust Antibody and T Cell Responses to SARS-CoV-2 in Patients with Antibody Deficiency. *J Clin Immunol*. 2021;41(6):1146-1153. doi:10.1007/s10875-021-01046-y

349. Hanitsch LG, Löbel M, Mieves JF, et al. Cellular and humoral influenza-specific immune response upon vaccination in patients with common variable immunodeficiency and unclassified antibody deficiency. *Vaccine*. 2016;34(21):2417-2423. doi:10.1016/j.vaccine.2016.03.091

350. Durkee-Shock JR, Keller MD. Immunizing the imperfect immune system: Coronavirus disease 2019 vaccination in patients with inborn errors of immunity. *Ann Allergy Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol*. Published online June 16, 2022:S1081-1206(22)00529-4. doi:10.1016/j.anai.2022.06.009

351. Delmonte OM, Bergerson JRE, Burbelo PD, et al. Antibody responses to the SARS-CoV-2 vaccine in individuals with various inborn errors of immunity. *J Allergy Clin Immunol*. 2021;148(5):1192-1197. doi:10.1016/j.jaci.2021.08.016

352. Healy K, Pin E, Chen P, et al. Salivary IgG to SARS-CoV-2 indicates seroconversion and correlates to serum neutralization in mRNA-vaccinated immunocompromised individuals. *Med N Y N*. 2022;3(2):137-153.e3. doi:10.1016/j.medj.2022.01.001

353. van Leeuwen LPM, GeurtsvanKessel CH, Ellerbroek PM, et al. Immunogenicity of the mRNA-1273 COVID-19 vaccine in adult patients with inborn errors of immunity. *J Allergy Clin Immunol*. 2022;149(6):1949-1957. doi:10.1016/j.jaci.2022.04.002

354. Abo-Helo N, Muhammad E, Ghaben-Amara S, Panasoff J, Cohen S. Specific antibody response of patients with common variable immunodeficiency to BNT162b2 coronavirus disease 2019 vaccination. *Ann Allergy Asthma Immunol Off Publ Am Coll Allergy Asthma Immunol*. 2021;127(4):501-503. doi:10.1016/j.anai.2021.07.021

355. Peet CJ, Papadopoulou C, Sombrito BRM, Wood MR, Lachmann HJ. COVID-19 and autoinflammatory diseases: prevalence and outcomes of infection and early experience of vaccination in patients on biologics. *Rheumatol Adv Pract*. 2021;5(2):rkab043. doi:10.1093/rap/rkab043

Table 1: Table shows vaccine types, specific target epitopes and their immunological responses.

S. No	Classification	Vaccine type	Epitope	Immunological response	Reference
1	**Component vaccines**	Protein subunit vaccine	S1, RBD	- Induces high level of neutralizing antibodies. - Induces humoral immune response.	127, 128
		Viral (Replicating & Non-replicating) vector vaccine	S, N	- Induces neutralizing antibodies & humoral immunity. - Induces $CD8^+$ T cell immune responses.	190, 191
		Nucleic acid (DNA & RNA) vaccine	S, S1	- Encodes neutralizing antibodies against S & S1 viral protein components. - Later on, induces cellular immune responses.	13-16, 37-41
2	**Whole virus vaccines**	Inactivated vaccine	S, N, M	- Induces high level of neutralizing antibodies. - Induces humoral immune response.	226-229
		Live-attenuated vaccine	S	- Induces cross-neutralizing antibodies. - Induces both cellular & humoral immunity.	275-278

Abbreviations: S: spike protein; N: Nucleo-capsid protein; M: Membrane protein; RBD: Receptor binding domain; and S1: Spike protein S1 subunit.

Table 2. List of clinically approved vaccine candidates with their manufacturer, pharmacological, immunological, and clinical details. It shows the brief information related to vaccine developer, the number of countries which have authorized its use, type, targeted antigen, dosage, route of administration, resulting efficacy, immunological responses, and ongoing registered clinical trial details of 28 vaccines approved till December 2021.

Sl. No	Vaccine (Countries app.) Type & Developer	Target	Booster dose (Day)	Efficacy	Immunological features	Ongoing registered clinical trial number	Ref.
1	ZF2001**(3)** • Protein Subunit • Anhui Zhifei Longcom	RBD	28 & 56	82%, (Alpha: 93%, Delta: 78%)	↑ in Th1, Th2 responses & GMT	**Phase-I:** NCT04445194, NCT04636333, NCT04550351 & NCT04961359 **Phase-II:** NCT04466085, NCT04813562 & NCT05109598 **Phase-III:** NCT04646590, ChiCTR2100050849, NCT05091411 & NCT05128643	136, 137
2	Covaxin **(12)** • Inactivated • Bharat Biotech	Whole virion	28	Symptomatic: 77.8 Asymptomatic: 63.6; Severe:93.4%	↑ in GMT, S1 protein, RBD, & N- protein.	**Phase-I:** NCT04471519 & CTRI/2020/09/027674 **Phase-II:** NCT04918797, NCT04471519 & CTRI/2020/09/027674 **Phase-III:** NCT04918797, NCT04641481	247, 248
3	Ad5-nCoV **(10)** • NRVVV • CanSino	Spike	Single	Moderate: 65.7% Severe 91%	↑ in spike receptor & GMT	**Phase-I:** NCT05043259, NCT04313127, NCT04568811 & NCT04840992 **Phase-II:** NCT05043259, NCT05005156, NCT04840992, NCT04341389 & NCT04566770 **Phase-III:** NCT04526990, NCT04540419	194, 195
4	CIGB-66 **(4)** • Protein Subunit • CIGB	RBD	14 & 28	92.28%	N/A	**Phase-I & II**: RPCEC00000345 & RPCEC00000346 **Phase-III:** RPCEC00000359	138, 139
5	KoviVac **(1)** • Inactivated • Chumakov Center	Whole virion	14	N/A	N/A	**Phase-I & II:** 502	255, 256
6	EpiVacCorona **(2)** • Protein Subunit • FBRI	Spike	21	N/A	Seroconversion (≥ 1:20)	**Phase-I & II:** NCT04527575 **Phase 3:** NCT04780035	134, 135
7	Sputnik Light **(21)** • NRVVV • Gamaleya	Spike	Single	Non-hospitalized: 79%; Hospitalized: 88% & Death: 85%	↑ in GMT & neutralizing Ab	**Phase-I:** NCT04713488 **Phase-II:** NCT04713488 & NCT05027672 **Phase-III:** NCT04741061	198, 199

8	Sputnik V **(74)** • NRVVV • Gamaleya	Spike	21	91.6%	↑ in both cellular and humoral immune responses	**Phase-I:** NCT04760730, NCT04684446, NCT04436471, 241 & NCT04437875 **Phase-II:** NCT05027672, NCT04988048, NCT04760730, NCT04954092, NCT04962906, NCT04983537, NCT04684446, NCT04686773, NCT04436471, 241, NCT04437875, NCT04587219 & NCT04640233 **Phase-III:** NCT04954092, NCT04640233, NCT04564716, NCT04530396 NCT04642339 & NCT04656613	196, 197
9	Soberana 02 **(3)** • Protein Subunit • IFVC	RBD	21	Two doses: 62% Booster: 91.2%	↑ in IFN−Y, IL-4, seroconversion, and anti-RBD Ab	**Phase-I:** IFV/COR/06 **Phase-II:** IFV/COR/08 **Phase-III:** IFV/COR/09	140, 141
10	Soberana Plus **(1)** • Protein Subunit • Instituto Finlay de Vacunas Cuba	Dimeric RBD	Single	91.2%	↑ in ACE2 interaction and GMT.	**Phase-I:** IFV/COR/05 & IFV/COR/15 **Phase-II:** IFV/COR/11 & IFV/COR/15 **Phase-III:** IFV/COR/09	141, 142
11	Ad26.COV2.S **(85)** • NRVVV • Johnson & Johnson	Spike	56	66.9%	↑ in S-protein and GMT.	**Phase-I:** NCT04889209, NCT05109559, NCT04509947, NCT04894305 & NCT04436276 **Phase-II:** NCT04889209, NCT05109559, NCT04436276, NCT04535453 & NCT04765384 **Phase-III:** NCT05048940, NCT05047640, NCT04505722, NCT04614948, NCT04838795 & NCT05091307	200, 201
12	QazVac **(2)** • Inactivated • RIBSP	Whole virion	21	N/A	↑ in Th1 response and GMT.	**Phase-I & II:** NCT04530357 **Phase-III:** NCT04691908	257, 258
13	MVC-COV1901 **(1)** • Protein subunit • Medigen	Spike (S2)	29	N/A	↑ in seroconversion and GMT.	**Phase-I:** NCT04487210 **Phase-II:** NCT04695652, NCT04822025, NCT04951388, NCT05038618, NCT05048849 & NCT05054621 **Phase-III:** NCT05011526	143, 144
14	SARS-CoV-2 Vaccine **(2)** • Inactivated • Minhai Biotech.	Whole virion	14 or 28	N/A	↑ in IFN-γ and T-cell responses, GMT & RBD Ab.	**Phase-I:** NCT04758273 & NCT05003479 **Phase-II:** NCT04756323 & NCT05003466 **Phase-III:** NCT04852705	259, 260

15	mRNA-1273 **(79)** • RNA • Moderna	Modifie RNA of Spike	28	94.1%	↑ in CD4+ T cell response	**Phase-I:** NCT04785144, NCT04813796, NCT04889209, NCT04839315, NL9275 & NCT04283461 **Phase-II:** NCT05027672, ISRCTN73765130, NCT04889209, NCT04894435, NCT04761822, NCT04847050, NCT04796896, NCT04930770, NCT04969263, NCT04988048, NL9275, NCT05022329, NCT05077254, NCT04405076, NCT04748471, NCT04649151 & EUCTR2021-002348-57 **Phase-III:** NCT05119855, NCT04805125, NCT04796896, NCT04811664, NCT04806113, NCT04860297, NCT05022329, NCT05048940 & NCT04649151	74, 75
16	FAKHRAVAC (MIVAC) **(1)** • Inactivated • ODIR	Whole virion	21	N/A	N/A	**Phase-I:** IRCT20210206050259N1 **Phase-II:** IRCT20210206050259N2 **Phase-III:** IRCT20210206050259N3	261, 262
17	AZD1222 **(127)** • NRVVV • Oxford & AstraZeneca		28 & 84	70.4% (Std. dose: 62.1% Low dose: 90%)	N/A	**Phase-I:** NCT04760730, NCT04684446, TCTR20211102006, NCT05133609, NCT04444674, PACTR202005681895696, NCT04816019, NCT04324606 & NCT04568031 **Phase-II:** NCT04973449, NCT05027672, ISRCTN73765130, CTRI/2020/08/027170, NCT05087368, NCT05054621, NCT04894435, NCT04988048, NCT04885764, EUCTR2021-002348-57, NCT04760730, NCT04962906, NCT04983537, NCT04684446, NCT04686773, NCT04992182, NCT05049226, TCTR20211102006, NCT04860739, EUCTR2021-001978-37, NCT04907331, ISRCTN69254139, NCT04998240, NCT04444674, PACTR202005681895696, NCT05059106, TCTR20211004005, NCT04324606, NCT04568031, ISRCTN15638344 & NCT04400838 **Phase-III:** NCT04973449, NCT05007951, NCT04864561, CTRI/2020/08/027170, NCT05011526, NCT04885764, NCT04800133, NCT05059106, EUCTR2020-001228-32, NCT04400838, ISRCTN89951424, NCT04536051, NCT04516746 & NCT04540393	202, 203

18	BNT162b2 **(112)** • RNA • Pfizer/BioNTech	RBD	21	95%	↑ in neutralizing antibody and CD8+/CD4+ T-cell responses	**Phase-I:** NCT04380701, NCT04889209, NCT04839315, NCT04969601, TCTR20211102006, NCT04816643, NCT04588480, NCT04936997 & EUCTR2020-005442-42 **Phase-II:** NCT04368728, NCT04949490, ISRCTN73765130, NCT04380701, NCT05077254, NCT04889209, NCT04894435, NCT04761822, NCT04969263, NCT05022329, EUCTR2021-002348-57, NCT04992182, NCT05049226, NCT04969601, EUCTR2021-005043-71, TCTR20211027002, TCTR20211102006, ISRCTN69254139, NCT04860739, NCT04907331, NCT04588480, NCT04649021, NCT04824638, NCT04895982, NCT04754594 & EUCTR2020-005442-42 **Phase-III:** NCT05124171, NCT04368728, NCT04805125, NCT05022329, NCT04800133, NCT04951323, NCT05047640, NCT04754594, NCT04713553, NCT04816669 & EUCTR2020-005442-42	81, 82
19	* Razi Cov Pars **(1)** • Protein Subunit • RVSRI	Spike	21 & 51	N/A	N/A	**Phase-I:** IRCT20201214049709N1 **Phase-II:** IRCT20201214049709N2 **Phase-III:** IRCT20201214049709N3	145, 146
20	Covishield **(47)** • NRVVV • Serum Institute	Spike	56 & 96	61% (Symptomatic: 76%)	Responses similar to ADZ1222	**Phase-II & III:** CTRI/2020/08/027170	203, 205
21	COVOVAX **(2)** • Protein Subunit • Serum Institute	Spike	21	CoV2 variants: UK: 86%; SA: 60% B.1.1.7: 89.3% B.1.351: 49.4%	↑ in GMT, IFN-γ, IL-2 TNF-α and CD4+ T-cell response	**Phase-II & III:** CTRI/2021/02/031554	147, 148
22	COVID-19 Inactivated Vaccine **(1)** • Inactivated • Shifa Pharmed	Whole virion	28	N/A	93.5% response in Ab production	**Phase-I:** IRCT20201202049567N1, IRCT20201202049567N2 & IRCT20171122037571N3 **Phase-II:** IRCT20201202049567N3 & IRCT20171122037571N3 **Phase-III:** IRCT20201202049567N3	263, 264
23	BBIBP-CorV **(72)** • Inactivated • Sinopharm	Whole virion	21	79%	↑ in neutralising Ab	**Phase-I:** IRCT20171122037571N3, NCT05109559 & ChiCTR2000032459 **Phase-II:** IRCT20171122037571N3, NCT04988048, NCT04962906, NCT04983537, NCT05109559, ChiCTR2000032459, NCT04998240 & TCTR20210920005 **Phase-III:** IRCT20210206050259N3, IRCT20201214049709N3, NCT04612972, ChiCTR2000034780, NCT04510207, NCT04560881, NCT04917523 & NCT04984408	249, 250

24	Inactivated (Vero Cells) **(2)** • Inactivated • Sinopharm	Whole virion	21	Symptomatic: 72.8% Severe: 100%	Seroconversion rate: 99.3% & GMT: 94.5	**Phase-I:** ChiCTR2000031809 **Phase-II:** NCT04885764 & ChiCTR2000031809 **Phase-III:** NCT04885764, NCT04612972, ChiCTR2000034780, NCT04510207 & ChiCTR2000039000	249, 251
25	CoronaVac **(47)** • Inactivated • Sinovac	Whole virion	14	84% (Symptomatic: 50.7% Mild cases: 83.7%)	Seroconversion: 95.6% (adults) & 87.5% (elderly) S1-RBD Ab: 96% (adults) & 100% (elderly).	**Phase-I:** NCT05043259, NCT05109559, NCT04352608, NCT04383574 & NCT04551547 **Phase-II:** NCT04979949, NCT05087368, NCT04884685, PHRR210210-003308, NCT04992182, NCT05049226, NCT05043259, NCT05109559, NCT04352608, NCT04383574 & NCT04551547 **Phase-III:** NCT05137418, NCT05077176, NCT04942405, NCT04800133, PHRR210210-003308, NCT04617483, NCT04651790, NCT04992260, NCT04456595, NCT04508075 & NCT04582344	252-254
26	TAK-919 **(1)** • RNA • Takeda	Modifie RNA of Spike	28	N/A	↑ in neutralizing Ab & CD4+ T-cell response.	**Phase-I & II:** NCT04677660	79, 80
27	COVAX-19 **(1)** • Protein Subunit • Vaxine/CinnaGen	Spike	21	N/A	↑ in neutralizing Ab & T cells response	**Phase-I:** NCT04453852 **Phase-II:** IRCT20150303021315N23 & NCT04944368 **Phase-III:** NCT05005559	149, 150
28	** ZyCoV-D **(1)** • DNA • Zydus Cadila	Spike	28 & 56	66.6%	Robust cellular and humoral immunity	**Phase-I & II:** CTRI/2020/07/026352 & CTRI/2021/03/032051 **Phase-III:** CTRI/2021/01/030416	30, 31

Abbreviations:
RBD: Receptor binding domain; Th1: T helper type 1; Th2: T helper type 2; GMT: Geometric mean titer; S1: Spike 1 protein; FBRI: Federal Budgetary Research Institution; IFN: Interferon; IL: Interleukin; ACE2: Angiotensin-1 converting enzyme 2; S-2P: Spike 2 protein; CIGB: Center for Genetic Engineering and Biotechnology; ODIR: Organization of Defensive Innovation and Research; IFVC: Instituto Finlay de Vacunas Cuba; RVSRI: Razi Vaccine and Serum Research Institute; NRVVV: Non Replicating Viral Vector Vaccines; and SA: South Africa.

Table 3. Type of adjuvants used in anti-SARS-CoV-2 vaccines development.

Classification	Adjuvants	Vaccine type	Route	Mechanism	Technology	Ref.
Aluminum	2% Alhydrogel	*Inactivated vaccines* (BIV1-CovIran, CoronaVac) *Subunit vaccines* (RBD)	IM, ID	Activate pro-inflammatory NLPR3 pathway and stimulate prime Th2 cell response	Alum-stabilized PAPE preparation, alum nano-encapsulation	258, 285
STING agonist	CF501	*Subunit vaccines* (RBD-Fc)	IM	Activate STING, induces cellular and humoral immunity	Derivative designing to improve solubility, potency and side effects	286, 287
Manganese	Nano-manganese	*Subunit vaccines* (RBD)	IM	Enhance cGAMP production and its binding with STING	Nano Mn preparation by chemical engineering	288, 289
O/W emulsion	MF59, AS03	*Subunit vaccines* (S)	IM	Enhances DC recruitment, CD4+ T cell priming and humoral immunity	Surfactant used to make uniform mixture of both oil and water phase	290, 291
TLR agonist	TLR3, 4, 7/8, LR1/2 agonist	*Subunit vaccines* (RBD, RBD-Fc, S1, S) *Inactivated vaccines* (BBV152)	IM	Induces IFN, cytokines, chemokine, and humoral immunity	N/A	292-295
Cationic nanocarriers	Chitosan, PEI, DOTAP	*Subunit vaccines* (RBD)	IM, IN	Activate cytotoxic CD8+ T lymphocytes & CD4+ Th cells	N/A	296
Matrix-M1	N/A	*Subunit vaccines* (S)	IM	Activate cellular and humoral immunity	N/A	155, 297
Advax-SM	Advax™ & CpG55.2	*Subunit vaccines* (ECD of S protein)	IM	Activate CD8+ DC's, robust T cell responses	N/A	298

Abbreviations: PEI: polyethyleneimine; TLR: toll-like receptor; NLPR3: NOD-like protein receptor 3; cGAMP: 20, 30-cyclic guanosine mono-phosphate adenosine monophosphate; IFN: type I interferon; Th2: T helper 2; STING: stimulator of interferon genes; PAPE: Pickering emulsion; N/A: not available.

Table 4. Vaccination of Inborn Errors of Immunity (IEI) patients and their immunological responses.

IEI diagnosis	Patient #	Vaccine	Adaptive immunity	Cellular immunity	Ref.
CVID	17	BNT162B2	65%	NR	354
CVID, XLA, Ab deficiency	21	BNT162B2	86-95% anti-spike RBD Ab,	76%	350
CVID	17	BNT162B2	70.5%	82%	345
CVID, XLA, CID	78	BNT162B2	73%	NR	343
CVID	33	BNT162B2	33%	NR	350
CVID	30	BNT162b2 booster, ChAdOx1	83% after any booster 80% after mRNA booster	53% for ChAdOx1, 83% for mRNA	344
CVID	58	BNT162B2	34% post-vaccination 100% post-infection	1/9 post-vaccination, 0/3 convalescent	346
CVID	5	BNT162B2, mRNA-1273	80%	NR	347
CVID	28	Ad26.COV2.S, ChAdOx1 nCoV-19, BNT162B2, mRNA-1273,	71.4%	71%	350
CVID	18	BNT162B2, mRNA-1273, ChAdOx1	83% after any dose 50% neutralizing Ab	83%	348
CVID, SAD	25	BNT162B2, mRNA-1273,	73%	NR	349
CVID, XLA	47	BNT162B2	20%	70% CVID 83% XLA	350
CVID	1	BNT162B2	100%	NR	351
CVID, PAD, SAD, XLA, CID, thymoma	168	BNT162B2, ChAdOx1	55%	46.20%	347
CVID XLA, WAS	11	BNT162B2, mRNA-1273	91%	NR	350
CVID, XLA, CID, PAD, phagocytic defects	505	mRNA-1273	>80% in all cases	88% overall, 67% CVID	353
WAS	1	BNT162B2	100%	100%	352
MagT1	1	BNT162B2	100%	NR	353
Primary antibody deficiency	62	BNT162B2, mRNA-1273, Ad26.COV2.S	59.7% after primary dose, 14X higher after booster 2	NR	350
CVID, CID, SAD, XLA, CHH, CTLA4, CGD, WAS, ADA2, IFNGR1, STAT3 LOF	156	BNT162B2, mRNA-1273, ChAdOx1	67%	NR	354
SCID, APECED, CID, CVID, RAG, MagT1, RALD, STAT-3 LOF, WAS, WHIM, XLA	81	BNT162B2, mRNA-1273, Ad26.COV2.S	85%	NR	351
XLA, SAD, CVID, Good syndrome, ATP6AP1 & PIK3R1 deficiency	33	BNT162B2, mRNA-1273	48% anti-RBD Ab, 6% anti-ACE2 receptor activity	77% T-cell specific Ab	355
XLA, STAT3 LOF, CVID, NFKB1	26	BNT162B2	69%	73%	350

Abbreviations: PAD: Primary antibody deficiency; IEI: Inborn errors of immunity; APECED: Autoimmune poly-endocrinopathy-candidiasis-ectodermal dystrophy; CVID: Common variable immunodeficiency; XLA: X-linked agammaglobulinemia, WAS: Wiskott-Aldrich syndrome; CID: Combined immunodeficiency; RBD: Receptor binding domain; SAD: Specific antibody deficiency; SCID: Severe combined immunodeficiency; and NR: Not reported.

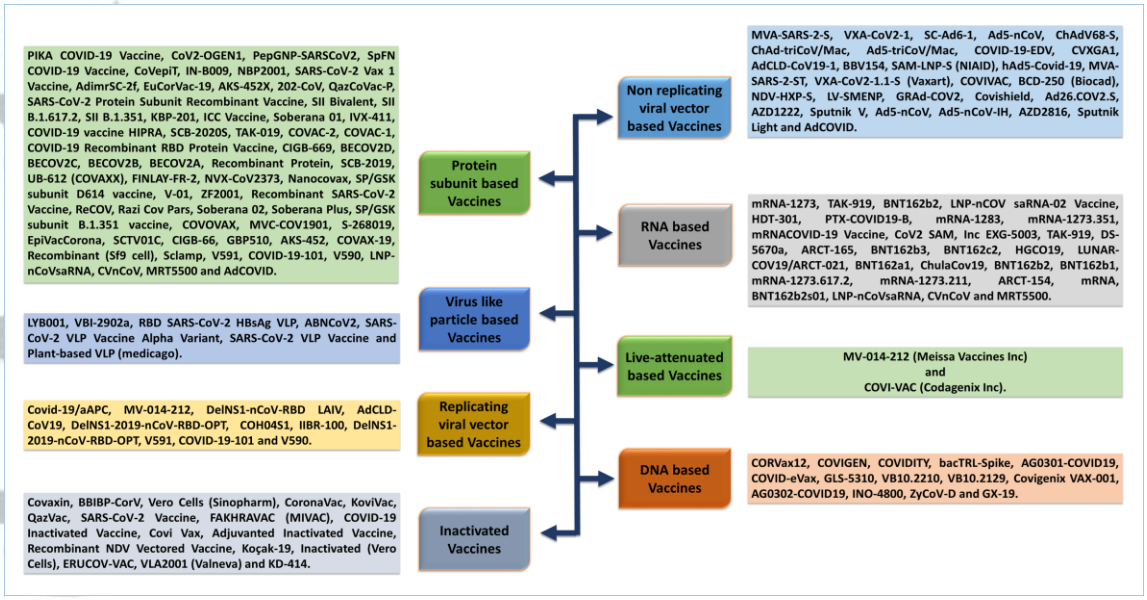

Fig. 1. Classification of SARS-CoV-2 vaccines. Figure depicts the classification of SARS-CoV-2 vaccines.

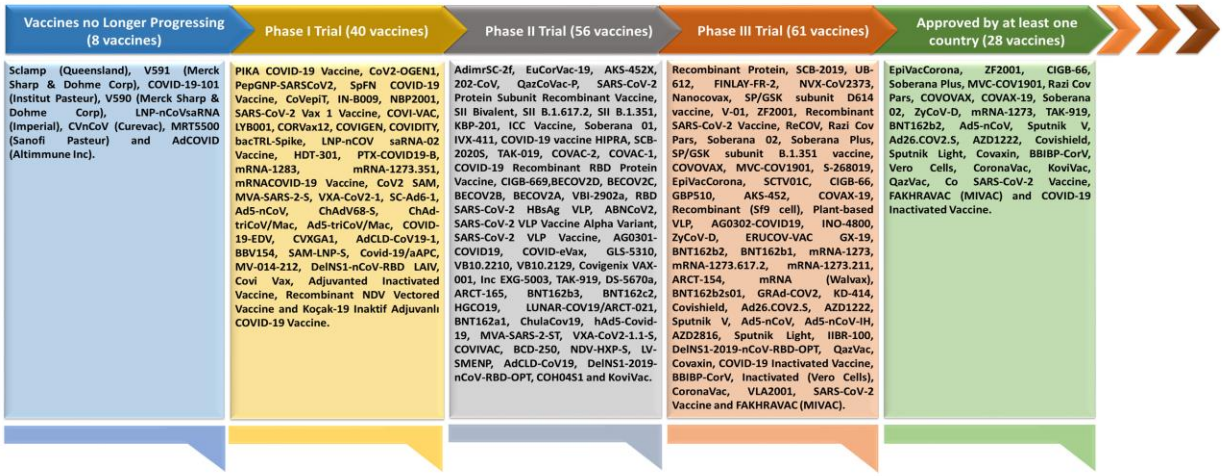

Fig. 2. Clinical Trial status of SARS-CoV-2 vaccines. Multiple panels of the figure shows the clinical status of vaccines such as 40 in phase-I, 56 in phase-II, 61 in phase-III and 28 out of them gets approved by at least one country for their emergency use. However, the 8 vaccines are removed from the clinical trials.