International Archives of Allergy and Immunology

Clinical Immunology – Review Article

Int Arch Allergy Immunol
DOI: 10.1159/000514225

Received: November 6, 2020
Accepted: December 30, 2020
Published online: February 1, 2021

Principles and Challenges in anti-COVID-19 Vaccine Development

Zuzana Strizova[a] Jitka Smetanova[a] Jirina Bartunkova[a] Tomas Milota[a, b]

[a]Department of Immunology, Second Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic; [b]Department of Paediatric and Adult Rheumatology, University Hospital Motol, Prague, Czech Republic

Keywords
Severe acute respiratory syndrome coronavirus 2 · Coronavirus disease 2019 · Vaccine · Clinical trials · *Bacillus* Calmette-Guérin · Measles

Abstract
The number of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-infected patients keeps rising in most of the European countries despite the pandemic precaution measures. The current antiviral and anti-inflammatory therapeutic approaches are only supportive, have limited efficacy, and the prevention in reducing the transmission of SARS-CoV-2 virus is the best hope for public health. It is presumed that an effective vaccination against SARS-CoV-2 infection could mobilize the innate and adaptive immune responses and provide a protection against severe forms of coronavirus disease 2019 (COVID-19) disease. As the race for the effective and safe vaccine has begun, different strategies were introduced. To date, viral vector-based vaccines, genetic vaccines, attenuated vaccines, and protein-based vaccines are the major vaccine types tested in the clinical trials. Over 80 clinical trials have been initiated; however, only 18 vaccines have reached the clinical phase II/III or III, and 4 vaccine candidates are under consideration or have been approved for the use so far. In addition, the protective effect of the off-target vaccines, such as *Bacillus* Calmette-Guérin and measles vaccine, is being explored in randomized prospective clinical trials with SARS-CoV-2-infected patients. In this review, we discuss the most promising anti-COVID-19 vaccine clinical trials and different vaccination strategies in order to provide more clarity into the ongoing clinical trials.

Introduction

The rapid spread of coronavirus disease 2019 (COVID-19) has globally become a serious issue [1]. The disease, caused by a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been first reported in December 2019 (Wuhan, China) and declared by the World Health Organization (WHO) as a pandemic on March 11, 2020 [2]. To date, the COVID-19-associated deaths keep rising in most of the European countries. This phenomenon can be influenced by the pandemic precaution measures, such as mandatory face masks wearing; however, the end of the pandemic may not be seen until an effective anti-COVID-19 vaccine is developed [3, 4]. SARS-CoV-2

Edited by: H.-U. Simon, Bern.

karger@karger.com
www.karger.com/iaa

Tomas Milota
Department of Immunology, 2nd Faculty of Medicine
Charles University and University Hospital Motol, V Úvalu 84
CZ–150 06 Prague (Czechia)
tomas.milota@fnmotol.cz

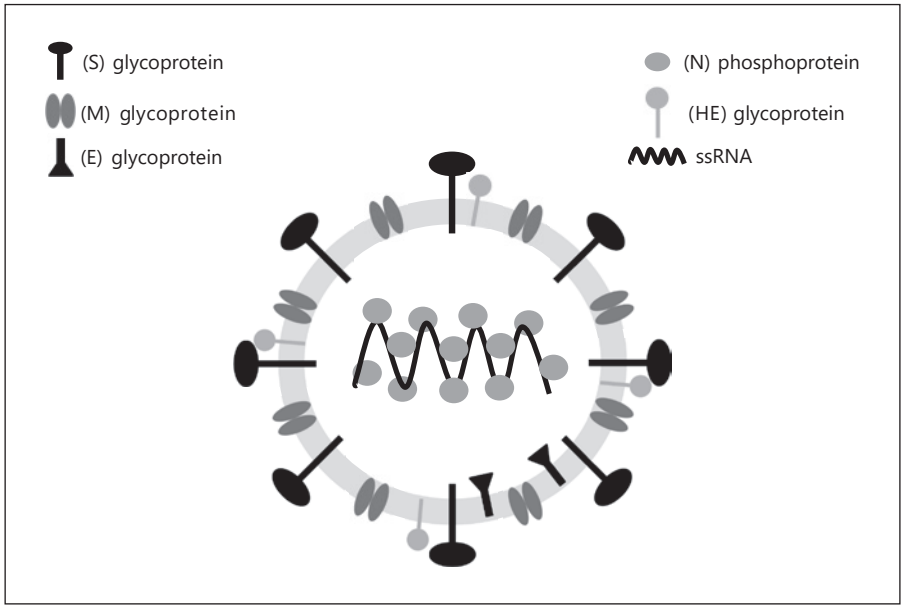

Fig. 1. Structure of SARS-CoV-2 virus: spike (S) glycoprotein, membrane (M) glycoprotein, envelope (E) glycoprotein, hemagglutinin-esterase (HA) glycoprotein, and nucleocapsid (N) phosphoprotein [97, 98].

belongs to the single-stranded RNA viruses which originated in bats [5]. SARS-CoV-2 exhibits the same structural and molecular patterns as other coronaviruses, such as structural proteins S (spike), E (envelope), M (membrane), and N (nucleocapsid) (Fig. 1) [6, 7]. The binding of the virus and its further entry into the host cell through angiotensin-converting enzyme 2 is mediated by the viral S protein. As the S protein is processed by a protease transmembrane protease serine 2, viral fusion with the host cell occurs. The first genetic analyses of the virus revealed an 89% nucleotide identity with bat virus SARS-like-CoVZXC21 [6]. Further investigation, however, confirmed a high similarity (96%) between the SARS-CoV-2 and the betaCoV RaTG13 of bats [1, 8, 9]. In COVID-19, it has been attempted by multiple studies to identify the intermediate host and on the basis of the available data, pangolins are most likely the mammals serving as a SARS-CoV-2 intermediate host [10]. The understanding and identification of an intermediate host is of major importance. The reason stems from the fact that blocking the human-host contact may restrain further spread of the novel disease variants, such as the Cluster 5 identified originally in minks. This cluster includes Y453F mutation in the spike protein and was reported among people in Denmark. Additionally, the virus was shown to infect multiple animal species under experimental conditions, and also few cases of household cats and dogs were reported to be positive for SARS-CoV-2 RNA. Therefore, concerns regarding the new sources of infection, novel potential viral strains, and uncontrolled outbreaks rise [11–13]. Moreover, the identification of an intermediate host may allow novel vaccine testing [1]. As a matter of fact, in Middle East respiratory syndrome disease, the first vaccine candidates were tested in dromedary camels [14]. The total number of deaths in COVID-19 is affected by the high transmissibility (assessed by the basic reproduction number, R_0) [15]. COVID-19 is mainly transmitted through respiratory droplets from sneezing and coughing; however, few other transmission routes have been described, such as alimentary transmission or through conjunctival mucosa [1, 4]. To date, severe and deadly forms of COVID-19 have been reported. Underlying health conditions were seen in cases with life-threatening course of COVID-19, and thus, the COVID-19 pandemic represents an enormous threat for the elderly [16] or chronically ill patients, particularly for those suffering from severe obesity, CKD, diabetes, arterial hypertension, or asthma [17]. On the other hand, the viral load is presumably one of the factors that dictate the clinical course of the disease [18]. This may also be affected by the transmission route, and therefore, asymptomatic COVID-19 patients were reported throughout multiple studies [19, 20]. Currently, multiple therapeutic approaches are being applied to deal with the infection. However, these approaches are rather supportive, and the prevention in reducing the transmission is the best hope for public health [1, 5, 21]. We have reviewed the current status of all anti-COVID-19 vaccines that have reached the clin-

ical trials in humans. As the race for the effective and safe vaccine has begun, different strategies were introduced. In this review, we discuss the most promising anti-COVID-19 vaccine clinical trials and discuss different vaccination strategies in order to provide more clarity into the ongoing clinical trials.

Methods

We conducted a comprehensive review of the literature on the progress of anti-SARS-CoV-2 vaccine development preventing the rapid spread of COVID-19 disease. The vaccines that were registered until December 2020 in the Clinical Trials database by the National Library of Medicine at the US National Institutes of Health were reviewed [22]. The authors followed the proposed guidelines for biomedical narrative review preparation [23].

Immune Response to SARS-CoV-2 Virus

The immune system affects the severity of the COVID-19 disease [7]. SARS-CoV-2 infection has an impact on both innate and adaptive immune responses. It has been described that SARS-CoV-2 enters the human body through physical barriers, such as respiratory tract, oral mucosa, and conjunctival epithelium [24, 25]. The dendritic cells, macrophages, and neutrophils represent the first line of defense, and their functions may be promoted by the production of type I and III interferons by SARS-CoV-2-infected epithelial cells [26]. The adaptive T-cell- and B-cell-mediated immune responses are also presented in COVID-19 disease and, however, can be suppressed by SARS-CoV-2 [7]. In some cases, the innate immune cells may contribute to the excessive inflammation and, therefore, to the disease progression. The inability to reach control over the infection may result in dysregulated inflammatory responses that are potentially lethal. The IgM and IgG antibodies to SARS-CoV-2 are detectable within 1–2 weeks and began to decrease by 8 weeks [27]. Several studies also reported that IgA response peaks earlier than IgM [28]. The antibody response particularly leads to production of neutralizing antibodies to the S protein and to the nucleoprotein. S protein is also the main target of the majority of newly designed vaccines [29]. The magnitude of neutralizing antibodies positively correlates with the disease severity and the robustness of T-cell response [30]. T-cell responses were detectable in individuals recovering from mild COVID-19 who did not have detectable antibody responses to SARS-CoV-2 [31, 32]. The effective vaccination may not eradicate the SARS-CoV-2 virus but may at least protect from severe and deadly forms of the COVID-19 disease [7]. Current knowledge regarding the diverse aspects of SARS-CoV-2-immune system interplay shall be reflected in the vaccine design, including the selection of antigens, the vaccine platforms and adjuvants, the vaccination routes, and the dosage regimens [33, 34]. The key points of the SARS-CoV-2 vaccination strategies are discussed below. To date, over 80 clinical trials have been registered in the Clinical Trials database by the National Library of Medicine at the US National Institutes of Health; however, only 34 of them are active and recruiting (11 of phase I, 8 of phase I/II, 3 of phase II, 1 of phase II/III, and 11 of phase III) [22]. Moreover, 2 vaccine candidates have been approved for use by the US *Food and Drug Administration* (FDA) – BNT162/Comirnaty and mRNA-1273). BNT162/Comirnaty has been also permitted by the *European Medicines Agency* (EMA). The vaccination program with BNT162/Comirnaty has been recently initiated in many European countries. The main features of the registered and ongoing anti-SARS-CoV-2 vaccine clinical trial are summarized in Table 1 (Phase I and I/II) and Table 2 (Phase II, II/III, and III).

Inactivated Vaccines

Inactivated vaccines are based on presenting the form of pathogen with a loss of disease-producing capacity. The virus cultivation occurs in cell lines that represent a substrate for the production of large quantities of antigen. Virus multiplication is often followed by a purification and concentration prior to the vaccine inactivation [35]. Formaldehyde and beta-propiolactone are used in the majority of licensed human antiviral vaccines to inactivate the virus [36]. Multiple doses or adjuvants are required to achieve sufficient efficacy of inactivated vaccines [37]. To date, 4 inactivated vaccines have reached the phase III clinical trials and are currently under evaluation (#NCT04510207, #NCT04508075, and #NCT04456595).

Subunit Vaccines

Subunit vaccines are composed of purified antigens instead of whole microorganisms, and different carriers serve as a transporter for those antigens. In the anti-SARS-CoV-2 subunit vaccines, the antigens are represented by viral proteins, peptides, or nanoparticles. Because of relatively low immunogenicity of the subunit vaccines, adjuvants are required to create a stronger immune response [38]. Currently, aluminum salts, virosomes, AS03 (α-tocopherol, surfactant polysorbate 80, and squalene), AS04 (*Monophosphoryl lipid A,* MPLA) and MF59 (squalene) are the most widespread licensing adjuvants [39, 40]. These adjuvant systems are also used in a number of anti-SARS-CoV-2 vaccines; however, novel adjuvants are tested as well. Advax-SM (clinical trial #NCT04453852) is an adjuvant composed of polysaccharide delta-inulin and CpG oligodeoxynucleotide (CpG ODN). CpG ODN is a TLR 9 agonist with T-helper 1 skewing properties [41]. Granulocyte macrophage colony-stimulating factor is a proinflammatory cytokine which may also serve as an adjuvant (#NCT03305341 and #NCT04386252).

Nonetheless, subunit vaccines provide a high level of safety. Bacterial expression systems represent the most commonly used technique to produce recombinant proteins with high expression. However, in antigens where posttranslational modification is required, the use of mammalian or insect cells may be considered [42]. Other offered alternatives include transgenic plants [43]. This technology has been also adopted as a source of SARS-CoV-2 virus spike protein for the purpose of vaccine development (phase I/II trial #NCT04473690). To date, there are no SARS-CoV-2-recombinant vaccines tested in phase III. Three vaccines are being evaluated in clinical phase I/II (#NCT04527575 and #NCT04537208) and phase II (#NCT04533399). Recombinant technologies, including bacterial, insect, or mammalian cell-based expression systems can also be used for the generation of virus-like particles (VLPs). VLPs that are formed by a capsid protein do not contain infectious viral RNA or DNA. Moreover, the antisense RNA can inhibit virus expression, and the viral RNA/DNA may activate different pattern recognition receptors and trigger antiviral immune responses. These responses are primarily characterized by a pro-

Table 1. Summary of active and recruiting Phase I and I/II trials registered in the Clinical Trials database (National Library of Medicine at the US National Institutes of Health)

Vaccine name	Mechanism of action	Trial design	Outcome	Dosage regimen	Participants, *n*	Estimated study completion	Sponsor	Identifier
Phase I clinical trials								
SCB-2019	Recombinant protein with adjuvant AS03 or CpG/alum	Randomized, double-blinded, placebo-controlled	Safety and immunogenicity	2 i.m. doses	150 patients (18–75 yr)	March, 2021	Clover Biopharmaceuticals AUS Pty Ltd	NCT04405908
AdimrSC-2f	Baculovirus vector with/ without alum	Open-label	Safety and immunogenicity	1 dose	70 patients (20–60 yr)	March, 2021	Adimmune Corporation	NCT04522089
MVA-SARS-2-S	Modified vaccinia virus ankara vector	Open-label	Safety, tolerability and immunogenicity	2 doses	30 patients (18–55 yr)	May, 2021		NCT04569383
MVC-COV1901	Reombinant protein with adjuvant CpG1018	Open-label	Safety and immunogenicity	2 i.m. dose	45 patients (20–50 yr)	June, 2021	Medigen Vaccine Biologics Corp.	NCT04487210
COVAX-19	Recombinant protein with adjuvant Advax-SM	Randomized, controlled	Safety and immunogenicity	1 dose	40 patients (18–45 yr)	July, 2021	Vaxine Pty Ltd	NCT04453852
GRAd-COV2	Gorilla Adenovirus vector	Open-label	Safety and immunogenicity	1 i.m. dose	90 patients (18–85 yr)	July, 2021	ReiThera Srl	NCT04528641
UB-612	Recombinant protein	Open-label	Safety, tolerability and immunogenicity	1 dose	60 patients (20–55 yr)	August, 2021	United Biomedical Inc., Asia	NCT04545749
SARS-CoV-2 Sclamp	Recombinant protein with adjuvant MF59	Randomized, double-blinded, placebo-controlled	Safety and immunogenicity	2 doses	216 patients (>56 yr)	September, 2021	The University of Queensland	NCT04495933
TMV-083	Measles vector	Randomized placebo-controlled and dose escalation	Safety, tolerability and immunogenicity	1–2 i.m. dose(s)	90 patients (18–55 yr)	October, 2 021	Institut Pasteur	NCT04497298
CoVac-1	Recombinant peptide with adjuvant XS15	Open label	Safety and immunogenicity	single s.c. dose	36 patients (15–≥75 yr)	December, 2021	University Hospital Tuebingen	NCT04546841
bacTRL-Spike	DNA (*Bifidobacterium longum* vector)	Observer-blinded, randomized, placebo-controlled	Safety, tolerability and immunogenicity	1 p.o. dose	12 patients (>18 yr)	February, 2022	Symvivo Corporation	NCT04334980
Phase I/II clinical trials								
ARCT-021	mRNA	Randomized, double-blinded, placebo-controlled	Safety, tolerability and immunogenicity	1 i.m. dose	92 patients (21–80 yr)	January, 2021	Arcturus Therapeutics, Inc.	NCT04480957
SARS-CoV-2	Inactivated virus	Randomized, double-blinded, placebo-controlled	Safety and immunogenicity	2 doses	552 patients (3–17 yr)	September, 2021	Sinovac Research and Development Co., Ltd.	NCT04551547
SARS-CoV-2	Inactivated virus	Randomized, double-blinded, placebo-controlled	Safety and immunogenicity	2 doses	942 patients (18–59 yr)	September, 2021	Chinese Academy of Medical Sciences	NCT04412538

Table 1 (continued)

Vaccine name	Mechanism of action	Trial design	Outcome	Dosage regimen	Participants, *n*	Estimated study completion	Sponsor	Identifier
Recombinant new coronavirus	Recombinant protein	Randomized, double-blined, placebo-controlled	Safety and tolerability	1 i.m. dose	50 patients (>60 yr)	December, 2021	Anhui Zhifei Longcom Biologic Pharmacy Co., Ltd.	NCT04550351
INO-4800	DNA (plasmid vector pGX9501)	Open label	Tolerability, safety, and immunogenicity	2 i.d. doses	160 patients (19–64 yr)	February, 2022	International Vaccine Institute	NCT04447781
GX-19	DNA	Randomized, double-blind, placebo-controlled	Safety, tolerability, and immunogenicity	2 i.m. doses	210 patients (18–50 yr)	June, 2022	Genexine, Inc.	NCT04445389
LV-SMENP-DC	Lentivirus modified DC and antigen-specific CTLs	Open-label trial	Safety and efficacy	1 s.c. dose/1 infusion	100 patients (6 mo–80 yr)	December, 2024	Shenzhen Geno-Immune Medical Institute	NCT04276896
COVID-19/aAPC	Inactivated artificial APC upon lentivirus modification	Open label	Safety and immune reactivity	3 s.c. doses	100 patients (6 mo–80 yr)	December, 2024	Shenzhen Geno-Immune Medical Institute	NCT04276896

APC, antigen-presenting cell.

duction of type I interferons and proinflammatory cytokines [44]. VLPs-based anti-SARS-CoV-2 vaccine (#NCT04450004) is currently tested in phase I clinical trials.

DNA Vaccines

DNA vaccines deliver coronavirus's genes to the human cells. The vaccination principle depends on the DNA translocation into the cell nucleus where the transcription of the antigen is initiated and followed by a translation. DNA vaccines frequently use plasmids as vectors. Depending on the route of vaccine administration (intramuscular, intradermal, and subcutaneous), either myocytes or keratinocytes are addressed. Nonetheless, antigen-presenting cells residing close to the site of application can be transfected directly by DNA vaccines as well. In such cases, the expressed antigens are loaded onto MHC I and MHC II molecules due to the cross-priming potential [45]. The produced antigens are either released by exosomes or apoptotic bodies which lead to a recognition by antigen-presenting cells and further evolvement of humoral or cytotoxic immune responses. Different delivery devices are used to create a robust immune response [46, 47]. The main safety concerns imply a possible integration of transfected DNA into somatic and/or germ cells of the host. In such cases, a dysregulation of gene expression might occur and lead to various mutations. However, only extrachromosomal plasmids with a very low level of chromosomal integration are usually employed in the development of DNA vaccines. Furthermore, the majority of plasmids remain at the site of administration [48]. Three anti-SARS-CoV-2 DNA vaccines are currently in phase I/II of clinical assessment (#NCT04527081, #NCT04447781, and #NCT04445389).

RNA Vaccines

Messenger RNA (mRNA) vaccines were first tested in early 1990s; however, their use was limited because of their instability [49]. The mRNA encodes the genetic information to produce an antigen, and thus, RNA vaccines also lead to a production of coronavirus's proteins in vivo. The in vitro generation of an RNA vaccine includes a reaction of a DNA plasmid template and a recombinant RNA polymerase. In addition, a synthetic cap analog and a poly(A) tail are added to form a mature RNA sequence. The stabilization is further achieved by various transport systems (such as lipid nanoparticles, nano-emulsions, and cationic peptides) or methods enabling facilitated transfection (gene gun and electroporation). Conventional mRNA vaccines are based on the initiation of the transient antigen expression in the cytoplasm of the host cells. Another platform is represented by self-amplifying mRNA vaccines that contain both the genes coding the targeted antigen as well as the genes required for the self-replication (mostly RNA-dependent RNA polymerase) [50]. The conventional mRNA vaccines induce a prompt antigen expression, and the expressed antigens generate both humoral and cellular immune responses [51–54]. In self-amplifying mRNA vaccines, a delayed antigen expression may prevail and limit the efficacy of the vaccine. Yet, the self-amplifying mRNA vaccine platform reaches higher yields, and thus, an equivalent protection is conferred at much lower doses [55]. Regarding the safety profiles, the replicons of both abovementioned platforms are not capable of producing viral particles due to the lack of viral structural proteins. Moreover, neither conventional nor self-amplifying mRNA vaccines can integrate into the host genome. The mRNA-based vaccines were able to induce production of functional antibodies with neutralizing properties

Table 2. Summary of active and recruiting Phase II, II/III, and III trials registered in Clinical Trials database (National Library of Medicine at the US National Institutes of Health)

Vaccine name	Mechanism of action	Trial design	Outcome	Dosage regimen	Participants, *n*	Estimated study completion	Sponsor	Identifier
Phase II clinical trials								
CVnCoV	mRNA	Observer-blinded, multicenter, controlled	Safety, reactogenicity, and immunogenicity	1–2 i.m. doses	660 patients (>18 yr)	November, 2021	CureVac AG	NCT04515147
NVX-CoV2373	Recombinant protein with adjuvant matrix-M1	Observer-blinded, randomized, placebo-controlled	Efficacy, immunogenicity, and safety	2 i.m. doses	4,400 patients (18–64 yr)	November, 2021	Novavax	NCT04533399
Ad5-nCoV	Adenovirus-5 vector	Randomized, double-blinded, placebo-controlled	Safety and immunogenicity	2 i.m. doses	481 patients (>6 yr)	October, 2022	CanSino Biologics Inc.	NCT04566770
Phase II/III clinical trials								
BNT162	mRNA	Observer-blinded, randomized, placebo-controlled	Safety, tolerability, immunogenicity and efficacy	2 i.m. doses	43,998 patients (>12 yr)	January, 2023	BioNTech RNA Pharmaceuticals GmbH	NCT04368728
Phase III clinical trials								
CoronaVac	Inactivated virus	Randomized, double-blinded, placebo-controlled	Efficacy, safety and immunogenicity	2 doses	13,000 patients (>18 yr)	April, 2021	Health Institutes of Turkey	NCT04582344
SARS-CoV-2	Inactivated virus	Randomized, double-blinded	Non-inferiority of the commercial scale	2 doses	1,040 patients (>18 yr)	May, 2021	Sinovac Research and Development Co., Ltd.	NCT04617483
SARS-CoV-2	Inactivated virus	Randomized, double-blinded, placebo-controlled	Efficacy, safety, and immunogenicity	2 doses	6,000 patients (18–60 yr)	September, 2021	Universidad Perunana Cayetano Heredia	NCT04612972
ChAdOx1 nCoV-19	ChAdOx1 vector	Randomized, controlled	Efficacy, safety, and immunogenicity	1–2 doses	10,300 patients (>18 yr)	September, 2021	University of Oxford	NCT04536051
SARS-CoV-2	Inactivated virus	Randomized, double-blinded, parallel placebo-controlled	Efficacy, safety, and immunogenicity	2 doses	45,000 patients (>18 yr)	September, 2021	China National Biotec Group Company limited	NCT04510207
SARS-CoV-2	Inactivated virus	Observer-blinded, randomized, placebo-controlled	Efficacy, safety, and immunogenicity	2 i.m. doses	1,620 patients (18–59 yr)	September, 2021	PT Bio Farma	NCT04508075
Adsorbed COVID-19	Inactivated virus	Randomized, double-blinded, placebo-Controlled	Efficacy and safety in health-care professionals	2 doses	13,060 patients (18–59 yr)	October, 2021	Butantan Institute	NCT04456595
SARS-CoV-2 rS/ matrix-M1-adjuvant	Recombinant S protein	Randomized, observer-blinded, placebo-controlled	Efficacy and safety	2 doses	15,000 patients (18–84 yr)	January, 2022	Novovax	NCT04583995
Ad5-nCoV	Adenovirus-5 vector	Randomized, double-blinded, placebo-controlled	Efficacy, safety, and immunogenicity	1 i.m. dose	40,000 patients(>18 yr)	January, 2022	CanSino Biologics Inc.	NCT04526990
AZD1222	ChAdOx1 vector	Randomized, double-blinded, placebo-controlled	Efficacy, safety, and immunogenicity	2 i.m. doses	40,000 patients (>18 yr)	February, 2023	AstraZeneca	NCT04516746
Ad26.COV2.S	Adenovirus-26 vector	Randomized, double-blinded, placebo-controlled	Efficacy and safety	1 i.m. dose	60,000 patients (>18 yr)	March, 2023	Janssen Vaccines & Prevention B.V.	NCT04505722

in rabies, influenza, or Zika virus and represent also a promising vaccination strategy in the prevention against COVID-19 infection [56]. The efficacy and safety are being assessed in the ongoing phase II and phase III clinical trials (#NCT04515147, #NCT04368728, and #NCT04470427).

Viral Vector-Based Vaccines

Viral vector-based vaccines (VBVs) are constructed by *engineering a viral vector to carry coronavirus genes and slowly replicate in the host cells. The replication leads to the production of coronavirus proteins and a subsequent immune system activation.* Potential viral vectors include a broad spectrum of both DNA and RNA viruses, such as adenoviruses, parvoviruses (e.g., adeno-associated viruses), togaviruses (e.g., Semliki Forest virus), paramyxoviruses (e.g., measles virus, Newcastle disease virus or human parainfluenza virus), rhabdoviruses (e.g., vesicular stomatitis virus), and poxviruses (e.g., Modified Vaccinia Ankara). These viral vectors can be constructed as replicating or nonreplicating vectors [57]. The efficacy of VBV may be significantly affected by the preexisting immunity of the host. This can be avoided by the use of nonhuman or rare serotype vectors [58, 59]. The main safety concerns include the potential of viral genes to integrate into the host genome and uncontrolled replication. On the other hand, the high yield production supports the use of VBV particularly in the time of disease outbreaks [60]. In SARS-CoV-2 vaccine development, the most commonly used vectors are the adenoviral vectors, such as ChAdOx (#NCT04536051 and #NCT04516746), adenovirus type 5 (#NCT04564716, #NCT04540419, and #NCT04526990), and adenovirus type 26 (#NCT04564716 and #NCT04505722). All these vaccines are currently being evaluated in phase III clinical trials. However, lentivirus (#NCT04276896 and #NCT04428073), measles (#NCT04498247 and #NCT04497298), baculovirus (#NCT04522089), or MVA (#NCT04569383) are also being tested.

Routes of Administration

The route of administration is another crucial aspect that significantly affects the vaccine efficacy. Conventional vaccination approaches include mucosal and parenteral administration. Parenteral route generally includes intramuscular (IM), subcutaneous (SC), and intradermal (ID) application [61]. Due to an increased infiltration of dermis with DCs, the ID application initiates greater adaptive immune response than the IM application providing a significant dose sparing effect. However, improved efficacy is associated with a less favorable safety profile [62]. Mucosal vaccines including the intranasal and oral administration routes provide a number of advantages, particularly the avoidance of a needle application and a lower risk of systemic adverse events (AEs). Nevertheless, the systemic responses to mucosal vaccination are weaker as compared to parenterally administrated vaccinations [63]. The majority of vaccinations are administered intramuscularly. However, the intradermal administration (#NCT03305341 and #NCT04447781), oral administration (#NCT04334980), or combined administration (intramuscular and mucosal) of the vaccines (#NCT04552366) is being evaluated.

The Most Promising Anti-COVID-19 Vaccines

The first vaccine with favorable results was the ChAdOx1 nCoV-19 (also known as AZD1222, AstraZeneca/University of Oxford). This vaccine was evaluated in July 2020 in the phase I/II single-blind randomized trial with 1077 participants. The patients were exposed to 2 doses of recombinant adenovirus vaccine ChAdOx1 nCoV-19 in a 28-day interval. Neutralizing antibodies against SARS-CoV-2 spike protein were detected in 91% of patients after the first dose. The production of virus-specific antibodies peaked on day 28, and a robust T-cell response was also observed. Severe AEs were not reported [64]. The efficacy has been recently confirmed in a pooled interim analysis of 4 phase I/–III clinical trials. [65]. The preliminary results of a double-blind, randomized, placebo-controlled phase I/II trial with another vaccine candidate Ad26.COV2 (Janssen-Cilag International N.V.) were published in September 2020 (data published as a preprint). The study included >800 patients, and the seroconversion rate with the production of anti-spike protein-neutralizing antibodies was seen in 83–100% patients across the cohorts. The specific T-helper 1 response was detected in 80–83% of the participants with a robust activation of CD8+ T cells. Local and systemic AEs included fever, headache, myalgia, and injection site pain. In November 2020, the preliminary results of an open-label clinical trial including 45 healthy adults treated with mRNA-1273 vaccine (Moderna Biotech Spain, S.L.) were shown. The vaccine was administered in 2 doses and various concentrations (25, 100, and 250 μg). The seroconversion occurred in all participants, and the response depended on the administered dose. On the other hand, higher doses were associated with increased risk of systemic AEs (reported in 33% participants) [66]. The mRNA BNT162 (Pfizer/BioNTech) vaccine has been proven in a large observer-blinded, randomized, placebo-controlled trial with >43,000 participants to be a safe and potent vaccine. Two doses (30 μg per dose) were administered in a 21-day interval. The overall reported efficacy of 95% was observed across different subgroups defined by age, sex, race, ethnicity, baseline BMI, and the presence of coexisting conditions. A 52% efficacy was observed after the first dose indicating early protection. Injection site reactions, fatigue, headaches, and fevers were the most common AEs (reported in 27% of patients) [67].

Off-Target Vaccinations

It has been shown that various vaccination principles bear a potential to prevent or at least to suppress the detrimental effect of COVID-19. The cross-protection has been discussed particularly in association with the *Bacillus* Calmette-Guérin (BCG) vaccination. In BCG vaccinated populations, the incidence and the severity of the COVID-19 disease appears to be lower than BCG-non-vaccinated populations [68–72]. A similar phenomenon of a cross-protection was also described in individuals after the measles infection, or the measles, mumps, rubella vaccination [73, 74]. These findings were supported by previous observations that a nonspecific effect of these vaccines protects against other infections including those of viral origin [75–78]. Therefore, a number of prospective randomized clinical trials has been initiated to validate the preventive effect of both BCG vaccine (#NCT04379336, #NCT04537663, #NCT04327206, #NCT04328441, #NCT04461379, #NCT04369794, #NCT04414267, #NCT04384549, and other) and the measles vaccine (NCT04357028 and NCT04475081).

Discussion

An effective vaccination against SARS-CoV-2 infection could mobilize the innate and adaptive immune responses and provide a protection against severe forms of COVID-19. Since the SARS-CoV-2 virus may undergo mutational changes and antigenically evolve over time, the vaccine may become, as in influenza, a seasonal protection. On the other hand, coronaviruses have a low mutation rate in comparison to other RNA viruses, particularly *Influenza type A* [79]. The anti-SARS-CoV-2 vaccination may not lead to the eradication of the disease, however, may most certainly decrease the disease-related mortality and morbidity [75, 80, 81].

In COVID-19, live vaccines have not yet been registered in human clinical trials. Previous studies have shown that booster (secondary) vaccination with life-attenuated viruses generate only limited immune response as compared to the first vaccination dose [82, 83]. Also, the preexisting immunity caused by previous COVID-19 infection may inhibit the efficacy of live attenuated vaccines and the presence of neutralizing antibodies can be associated with the virus neutralization. Moreover, the genome instability may lead to a back mutation recovering their virulence, mainly in viruses with higher mutation rate [84–86]. Therefore, the live vaccines may not represent the optimal vaccine type in prevention of COVID-19 infection [87].

Other classical vaccination approaches, such as inactivated or recombinant subunit vaccines, are currently being tested against COVID-19 infection in clinical trials. Their efficacy is, however, also limited by relatively low response rates and short-term immune memory. Therefore, both approaches require the use of potent adjuvants such as CpG ODN, ADVAX-SM, or granulocyte macrophage colony-stimulating factor representing novel strategies to enhance immune response. Another obstacle of inactivated vaccines represents the risk of reversed outcome associated with enhanced virus-mediated disease and fatal consequences. In the cases of respiratory syncytial virus vaccination, the vaccine was found to be immunogenic; however, the elicited antibodies were nonprotective and respiratory syncytial virus disease progression in single cases resulted in death in vaccinated infants [88, 89]. Therefore, novel vaccine designs, such as mRNA vaccines and recombinant VBV vaccines are being extensively investigated to avoid these barriers. FDA and EMA have been recently authorized mRNA vaccine candidate BNT162/comirnaty and mRNA-1273 for the use [90]. Furthermore, other vaccine candidates, including Ad26.COV2.S and ChAdOx1-SARS-CoV-2 (AZD1222), are currently under consideration [91]. To note, novel vaccine approaches raise many safety concerns. However, strategies following the good manufacturing practice principles and appropriate preclinical and clinical testing under the surveillance of regulatory authorities should ensure good safety profile [92]. The efficacy and safety also remain an issue in immunocompromised patients with primary or secondary immunodeficiencies. Generally, administration of attenuated life virus vaccines has to be considered with caution and indicated upon careful individual assessment of risk and benefits in these patients. Non-live vaccines such as influenza or pneumococcal vaccine are regarded as safe, even though their efficacy may be reduced in patients with severely impaired antibody response [93, 94]. Similar principles might be applied also in anti-SARS-CoV-2 vaccination; however, neither recommendations nor guidelines are available yet. The level of virus-specific antibodies does not correlate with the acquired immune response mediated by T cells that might be preserved in the majority of the antibody deficient patients [95, 96]. Thus, the examination of T-cell response should also be considered in healthy subjects. Surprisingly, other vaccines such as BCG and measles vaccine have also shown efficacy in the prevention of COVID-19 disease. The leading vaccine candidates are currently being distributed and among selected population with great expectations to reduce the COVID-19 spread.

Acknowledgements

The preparation of this review article was endorsed by the Department of Immunology, Second Faculty of Medicine, Charles University, University Hospital Motol, and Department of Paediatric and Adult Rheumatology, University Hospital Motol, Prague, Czech Republic.

Statement of Ethics

Ethics approval was not required.

Conflict of Interest Statement

Z.S., J.S., J.B., and T.M. declare that they have no conflict of interest regarding the publication of this article.

Funding Sources

The preparation of the manuscript was supported by internal financial sources of the Department of Immunology, Second Faculty of Medicine, Charles University, and University Hospital Motol. No external funding sources were required.

Author Contributions

Z.S.: substantial contributions to the conception and design of the manuscript, analysis and interpretation of the literature, and drafting. J.S.: substantial contributions to the analysis and interpretation of the literature and drafting. J.B.: revising the manuscript critically for important intellectual content and final approval of the version to be published. T.M.: substantial contributions to the conception and design of the manuscript, analysis and interpretation of the literature, drafting, and revising it critically for important intellectual content.

References

1 Weston S, Frieman MB. COVID-19: knowns, unknowns, and questions. mSphere. 2020; 5(2):e00203–20.
2 Hiscott J, Alexandridi M, Muscolini M, Tassone E, Palermo E, Soultsioti M, et al. The global impact of the coronavirus pandemic. Cytokine Growth Factor Rev. 2020;53:1–9.
3 Kaur SP, Gupta V. COVID-19 vaccine: a comprehensive status report. Virus Res. 2020; 288:198114.
4 Strizova Z, Bartunkova J, Smrz D. Can wearing face masks in public affect transmission route and viral load in COVID-19? Cent Eur J Public Health. 2020;28(2):161–2.
5 Prompetchara E, Ketloy C, Palaga T. Immune responses in COVID-19 and potential vaccines: lessons learned from SARS and MERS epidemic. Asian Pac J Allergy Immunol. 2020; 38(1):1–9.
6 Chan JF, Kok KH, Zhu Z, Chu H, To KK, Yuan S, et al. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microbes Infect. 2020;9(1):221–36.
7 Paces J, Strizova Z, Smrz D, Cerny J. COVID-19 and the immune system. Physiol Res. 2020;69(3):379–88.
8 Zhou H, Chen X, Hu T, Li J, Song H, Liu Y, et al. A novel bat coronavirus closely related to SARS-CoV-2 contains natural insertions at the S1/S2 cleavage site of the spike protein. Curr Biol. 2020;30(11):2196–203.e3.
9 Zheng J. SARS-CoV-2: an emerging coronavirus that causes a global threat. Int J Biol Sci. 2020;16(10):1678–85.
10 Lam TT, Jia N, Zhang YW, Shum MH, Jiang JF, Zhu HC, et al. Identifying SARS-CoV-2-related coronaviruses in Malayan pangolins. Nature. 2020;583(7815):282–5.
11 Johansen MD, Irving A, Montagutelli X, Tate MD, Rudloff I, Nold MF, et al. Animal and translational models of SARS-CoV-2 infection and COVID-19. Mucosal Immunol. 2020;13(6):877–91.
12 Mallapaty S. COVID mink analysis shows mutations are not dangerous: yet. Nature. 2020;587(7834):340–1.
13 Patterson EI, Elia G, Grassi A, Giordano A, Desario C, Medardo M, et al. Evidence of exposure to SARS-CoV-2 in cats and dogs from households in Italy. Nat Commun. 2020; 11(1):6231.
14 Haagmans BL, van den Brand JM, Raj VS, Volz A, Wohlsein P, Smits SL, et al. An orthopoxvirus-based vaccine reduces virus excretion after MERS-CoV infection in dromedary camels. Science. 2016;351(6268):77–81.
15 Petrosillo N, Viceconte G, Ergonul O, Ippolito G, Petersen E. COVID-19, SARS and MERS: are they closely related? Clin Microbiol Infect. 2020;26(6):729–34.
16 Daoust JF. Elderly people and responses to COVID-19 in 27 Countries. PLoS One. 2020; 15(7):e0235590.
17 Ko JY, Danielson ML, Town M, Derado G, Greenlund KJ, Daily Kirley P, et al. Risk factors for COVID-19-associated hospitalization: COVID-19-associated hospitalization surveillance network and behavioral risk factor surveillance system. Clin Infect Dis. 2020 Sep 18:ciaa1419.
18 Fajnzylber J, Regan J, Coxen K, Corry H, Wong C, Rosenthal A, et al. SARS-CoV-2 viral load is associated with increased disease severity and mortality. Nat Commun. 2020; 11(1):5493.
19 Gao Z, Xu Y, Sun C, Wang X, Guo Y, Qiu S, et al. A systematic review of asymptomatic infections with COVID-19. J Microbiol Immunol Infect. 2020.
20 He J, Ni Y, Liao L, Xiao Y, Guo Y. Proportion of asymptomatic coronavirus disease 2019: a systematic review and meta-analysis. J Med Virol. 2021 Feb;93(2):820–30.
21 Song Y, Zhang M, Yin L, Wang K, Zhou Y, Zhou M, et al. COVID-19 treatment: close to a cure? A rapid review of pharmacotherapies for the novel coronavirus (SARS-CoV-2). Int J Antimicrob Agents. 2020;56(2):106080.
22 National Library of Medicine at the U.S. National Institutes of Health. ClinicalTrials. Available from: www.clinicaltrials.gov.
23 Gasparyan AY, Ayvazyan L, Blackmore H, Kitas GD. Writing a narrative biomedical review: considerations for authors, peer reviewers, and editors. Rheumatol Int. 2011;31(11): 1409–17.
24 Catanzaro M, Fagiani F, Racchi M, Corsini E, Govoni S, Lanni C. Immune response in COVID-19: addressing a pharmacological challenge by targeting pathways triggered by SARS-CoV-2. Signal Transduct Target Ther. 2020;5(1):84.
25 Rizzo P, Vieceli Dalla Sega F, Fortini F, Marracino L, Rapezzi C, Ferrari R. COVID-19 in the heart and the lungs: could we "Notch" the inflammatory storm? Basic Res Cardiol. 2020; 115(3):31.
26 Mason RJ. Pathogenesis of COVID-19 from a cell biology perspective. Eur Respir J. 2020; 55(4):2000607.
27 Lou B, Li TD, Zheng SF, Su YY, Li ZY, Liu W, et al. Serology characteristics of SARS-CoV-2 infection after exposure and post-symptom onset. Eur Respir J. 2020;56(2):2000763.
28 Padoan A, Sciacovelli L, Basso D, Negrini D, Zuin S, Cosma C, et al. IgA-Ab response to spike glycoprotein of SARS-CoV-2 in patients with COVID-19: a longitudinal study. Clin Chim Acta. 2020;507:164–6.
29 Tai W, He L, Zhang X, Pu J, Voronin D, Jiang S, et al. Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. Cell Mol Immunol. 2020;17(6):613–20.
30 Ni L, Ye F, Cheng M-L, Feng Y, Deng Y-Q, Zhao H, et al. Detection of SARS-CoV-2-specific humoral and cellular immunity in COVID-19 convalescent individuals. Immunity. 2020;52(6):971–7.
31 Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. Cell. 2020;181(7):1489–1501.e15.
32 Sekine T, Perez-Potti A, Rivera-Ballesteros O, Strålin K, Gorin JB, Olsson A, et al. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. Cell. 2020;183(1):158–68.e14.
33 Jeyanathan M, Afkhami S, Smaill F, Miller MS, Lichty BD, Xing Z. Immunological considerations for COVID-19 vaccine strategies. Nat Rev Immunol. 2020;20(10):615–32.
34 Poland GA, Ovsyannikova IG, Kennedy RB. SARS-CoV-2 immunity: review and applications to phase 3 vaccine candidates. Lancet. 2020 Nov 14;396(10262):1595–606.
35 Plotkin S. History of vaccination. Proc Natl Acad Sci U S A. 2014;111(34):12283–7.

36 Delrue I, Verzele D, Madder A, Nauwynck HJ. Inactivated virus vaccines from chemistry to prophylaxis: merits, risks and challenges. Expert Rev Vaccines. 2012;11(6):695–719.
37 Zepp F. Principles of vaccine design-lessons from nature. Vaccine. 2010;28(Suppl 3):C14–24.
38 Vartak A, Sucheck SJ. Recent advances in subunit vaccine carriers. Vaccines. 2016 Apr 16; 4(2):12.
39 Bonam SR, Partidos CD, Halmuthur SKM, Muller S. An overview of novel adjuvants designed for improving vaccine efficacy. Trends Pharmacol Sci. 2017;38(9):771–93.
40 Di Pasquale A, Preiss S, Tavares Da Silva F, Garçon N. Vaccine adjuvants: from 1920 to 2015 and beyond. Vaccines. 2015;3(2):320–43.
41 Honda-Okubo Y, Barnard D, Ong CH, Peng BH, Tseng CT, Petrovsky N. Severe acute respiratory syndrome-associated coronavirus vaccines formulated with delta inulin adjuvants provide enhanced protection while ameliorating lung eosinophilic immunopathology. J Virol. 2015;89(6):2995–3007.
42 Nascimento IP, Leite LC. Recombinant vaccines and the development of new vaccine strategies. Braz J Med Biol Res. 2012;45(12): 1102–11.
43 Tiwari S, Verma PC, Singh PK, Tuli R. Plants as bioreactors for the production of vaccine antigens. Biotechnol Adv. 2009;27(4):449–67.
44 Syomin BV, Ilyin YV. Virus-like particles as an instrument of vaccine production. Mol Biol. 2019;53(3):323–34.
45 Hobernik D, Bros M. DNA vaccines-how far from clinical use? Int J Mol Sci. 2018;19(11): 3605.
46 Donnelly JJ, Wahren B, Liu MA. DNA vaccines: progress and challenges. J Immunol. 2005;175(2):633–9.
47 Li L, Petrovsky N. Molecular mechanisms for enhanced DNA vaccine immunogenicity. Expert Rev Vaccines. 2016;15(3):313–29.
48 Schalk JA, Mooi FR, Berbers GA, van Aerts LA, Ovelgönne H, Kimman TG. Preclinical and clinical safety studies on DNA vaccines. Hum Vaccin. 2006;2(2):45–53.
49 Ross J. mRNA stability in mammalian cells. Microbiol Rev. 1995;59(3):423–50.
50 Zhang C, Maruggi G, Shan H, Li J. Advances in mRNA vaccines for infectious diseases. Front Immunol. 2019;10:594.
51 Ablasser A, Poeck H, Anz D, Berger M, Schlee M, Kim S, et al. Selection of molecular structure and delivery of RNA oligonucleotides to activate TLR7 versus TLR8 and to induce high amounts of IL-12p70 in primary human monocytes. J Immunol. 2009;182(11):6824–33.
52 Hua Z, Hou B. TLR signaling in B-cell development and activation. Cell Mol Immunol. 2013;10(2):103–6.
53 Kato H, Oh SW, Fujita T. RIG-I-like receptors and type I interferonopathies. J Interferon Cytokine Res. 2017;37(5):207–13.
54 Pollard C, Rejman J, De Haes W, Verrier B, Van Gulck E, Naessens T, et al. Type I IFN counteracts the induction of antigen-specific immune responses by lipid-based delivery of mRNA vaccines. Mol Ther. 2013;21(1):251–9.
55 Vogel AB, Lambert L, Kinnear E, Busse D, Erbar S, Reuter KC, et al. Self-amplifying RNA vaccines give equivalent protection against influenza to mRNA vaccines but at much lower doses. Mol Ther. 2018;26(2):446–55.
56 Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines: a new era in vaccinology. Nat Rev Drug Discov. 2018;17(4):261–79.
57 Ramezanpour B, Haan I, Osterhaus A, Claassen E. Vector-based genetically modified vaccines: exploiting Jenner's legacy. Vaccine. 2016;34(50):6436–48.
58 Fausther-Bovendo H, Kobinger GP. Pre-existing immunity against Ad vectors: humoral, cellular, and innate response, what's important? Hum Vaccin Immunother. 2014;10(10): 2875–84.
59 Humphreys IR, Sebastian S. Novel viral vectors in infectious diseases. Immunology. 2018;153(1):1–9.
60 Rauch S, Jasny E, Schmidt KE, Petsch B. New vaccine technologies to combat outbreak situations. Front Immunol. 2018;9:1963.
61 Zhang L, Wang W, Wang S. Effect of vaccine administration modality on immunogenicity and efficacy. Expert Rev Vaccines. 2015; 14(11):1509–23.
62 Schnyder JL, De Pijper CA, Garcia Garrido HM, Daams JG, Goorhuis A, Stijnis C, et al. Fractional dose of intradermal compared to intramuscular and subcutaneous vaccination: a systematic review and meta-analysis. Travel Med Infect Dis. 2020 Sep– Oct;37:101868.
63 Li M, Wang Y, Sun Y, Cui H, Zhu SJ, Qiu HJ. Mucosal vaccines: strategies and challenges. Immunol Lett. 2020;217:116–25.
64 Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-Rammerstorfer S, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. Lancet. 2020; 396(10249):467–78.
65 Voysey M, Clemens SAC, Madhi SA, Weckx LW, Folegatti PM, Aley PK, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet. 2020 Dec 8;397(10269):99–111.
66 Jackson LA, Anderson EJ, Rouphael NG, Roberts PC, Makhene M, Coler RN, et al. An mRNA vaccine against SARS-CoV-2: preliminary report. N Engl J Med. 2020;383(20): 1920–31.
67 Polack FP, Thomas SK, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med. 2020 Dec 31;383(27): 2603–15.
68 Escobar LE, Molina-Cruz A, Barillas-Mury C. BCG vaccine protection from severe coronavirus disease 2019 (COVID-19). Proc Natl Acad Sci U S A. 2020;117(30):17720–6.
69 Kamat S, Kumari M. BCG against SARS-CoV-2: second youth of an old age vaccine? Front Pharmacol. 2020;11:1050.
70 Moorlag SJCFM, van Deuren RC, van Werkhoven CH, Jaeger M, Debisarun P, Taks E, et al. Safety and COVID-19 symptoms in individuals recently vaccinated with BCG: a retrospective cohort study. Cell Rep Med. 2020; 1(5):100073.
71 Rajarshi K, Chatterjee A, Ray S. BCG vaccination strategy implemented to reduce the impact of COVID-19: hype or hope? Med Drug Discov. 2020;7:100049.
72 Sharma A, Kumar Sharma S, Shi Y, Bucci E, Carafoli E, Melino G, et al. BCG vaccination policy and preventive chloroquine usage: do they have an impact on COVID-19 pandemic? Cell Death Dis. 2020;11(7):516.
73 Anbarasu A, Ramaiah S, Livingstone P. Vaccine repurposing approach for preventing COVID 19: can MMR vaccines reduce morbidity and mortality? Hum Vaccin Immunother. 2020;16(9):2217–8.
74 Ashford JW, Gold JE, Huenergardt MJA, Katz RBA, Strand SE, Bolanos J, et al. MMR vaccination: a potential strategy to reduce severity and mortality of COVID-19 Illness. Am J Med. 2020 Oct 23;S0002–9343(20):30902–5.
75 Aaby P, Roth A, Ravn H, Napirna BM, Rodrigues A, Lisse IM, et al. Randomized trial of BCG vaccination at birth to low-birth-weight children: beneficial nonspecific effects in the neonatal period? J Infect Dis. 2011;204(2): 245–52.
76 Moorlag SJCFM, Arts RJW, van Crevel R, Netea MG. Non-specific effects of BCG vaccine on viral infections. Clin Microbiol Infect. 2019;25(12):1473–8.
77 Rieckmann A, Villumsen M, Sørup S, Haugaard LK, Ravn H, Roth A, et al. Vaccinations against smallpox and tuberculosis are associated with better long-term survival: a Danish case-cohort study 1971–2010. Int J Epidemiol. 2017;46(2):695–705.
78 Sorup S, Benn CS, Poulsen A, Krause TG, Aaby P, Ravn H. Live vaccine against measles, mumps, and rubella and the risk of hospital admissions for nontargeted infections. JAMA. 2014;311(8):826–35.
79 Abdelrahman Z, Li M, Wang X. Comparative review of SARS-CoV-2, SARS-CoV, MERS-CoV, and influenza A respiratory viruses. Front Immunol. 2020;11:552909.
80 Lewnard JA, Cobey S. Immune history and influenza vaccine effectiveness. Vaccines. 2018 May 21;6(2):28.
81 Rosendahl Huber SK, Hendriks M, Jacobi RHJ, van de Kassteele J, Mandersloot-Oskam JC, van Boxtel RAJ, et al. Immunogenicity of influenza vaccines: evidence for differential effect of secondary vaccination on humoral and cellular immunity. Front Immunol. 2018; 9:3103.

82 Christenson B, Böttiger M. Measles antibody: comparison of long-term vaccination titres, early vaccination titres and naturally acquired immunity to and booster effects on the measles virus. Vaccine. 1994;12(2):129–33.
83 Kongsgaard M, Bassi MR, Rasmussen M, Skjødt K, Thybo S, Gabriel M, et al. Adaptive immune responses to booster vaccination against yellow fever virus are much reduced compared to those after primary vaccination. Sci Rep. 2017;7(1):662.
84 Hanley KA. The double-edged sword: how evolution can make or break a live-attenuated virus vaccine. Evolution. 2011;4(4):635–43.
85 Lauring AS, Jones JO, Andino R. Rationalizing the development of live attenuated virus vaccines. Nat Biotechnol. 2010;28(6):573–9.
86 Minor PD. Live attenuated vaccines: historical successes and current challenges. Virology. 2015;479–480:379–92.
87 Mok DZL, Chan KR. The effects of pre-existing antibodies on live-attenuated viral vaccines. Viruses. 2020 May 8;12(5):520.
88 Amanna IJ, Slifka MK. Wanted, dead or alive: new viral vaccines. Antiviral Res. 2009;84(2):119–30.
89 Delgado MF, Coviello S, Monsalvo AC, Melendi GA, Hernandez JZ, Batalle JP, et al. Lack of antibody affinity maturation due to poor Toll-like receptor stimulation leads to enhanced respiratory syncytial virus disease. Nat Med. 2009;15(1):34–41.
90 U.S. Food and Drug Administration. Accessed 2020 Dec 11. Available from: https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/covid-19-vaccines.
91 European Medicines Agency. Accessed 2020 Nov 6. Available from: https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/coronavirus-disease-covid-19/treatments-vaccines-covid-19.
92 Milstien J, Costa A, Jadhav S, Dhere R. Reaching international GMP standards for vaccine production: challenges for developing countries. Expert Rev Vaccines. 2009;8(5):559–66.
93 Bonilla FA. Update: vaccines in primary immunodeficiency. J Allergy Clin Immunol. 2018;141(2):474–81.
94 Furer V, Rondaan C, Heijstek MW, Agmon-Levin N, van Assen S, Bijl M, et al. 2019 update of EULAR recommendations for vaccination in adult patients with autoimmune inflammatory rheumatic diseases. Ann Rheum Dis. 2020;79(1):39–52.
95 Friedmann D, Goldacker S, Peter H-H, Warnatz K. Preserved cellular immunity upon influenza vaccination in most patients with common variable immunodeficiency. J Allergy Clin Immunol Pract. 2020;8(7):2332–40.
96 Mieves JF, Wittke K, Freitag H, Volk HD, Scheibenbogen C, Hanitsch LG. Influenza vaccination in patients with common variable immunodeficiency (CVID). Curr Allergy Asthma Rep. 2017;17(11):78.
97 Florindo HF, Kleiner R, Vaskovich-Koubi D, Acúrcio RC, Carreira B, Yeini E, et al. Immune-mediated approaches against COVID-19. Nat Nanotechnol. 2020;15(8):630–45.
98 Chams N, Chams S, Badran R, Shams A, Araji A, Raad M, et al. COVID-19: a multidisciplinary review. Front Public Health. 2020;8:383.