Principles and Challenges in anti-COVID-19 Vaccine Development

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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].
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 II/III, 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 #NCT0436252).

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 (#NCT04527757 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 DNA 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-
| 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 | Recombinant 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, 2021 | Institut Pasteur | NCT04497298 |
| CoVAcc-1 | Recombinant peptide with adjuvant XS15 | Open label | Safety and immunogenicity | single s.c. dose | 36 patients (15–275 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 | Participants, Estimated completion | Sponsor Identifier |
|--------------|---------------------|--------------|---------|--------|----------------------------------|-------------------|
| DNA (plasmid vector pCMV951) DNA | 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. | Randomized, double-blinded, placebo-controlled | Safety and tolerability and immunogenicity | 1 i.m. dose | 50 patients (>60 yr) | December, 2021 Anhui Zhifei Longcom Biologic Pharmacy Co., Ltd. NCT04550351 |
| DNA (plasmid vector pCMV951) DNA | 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. | Randomized, double-blinded, placebo-controlled | Safety and tolerability and immunogenicity | 2 i.d. doses | 160 patients (19–64 yr) | February, 2022 International Vaccine Institute NCT04447781 |
| GX-19 DNA | 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. | Randomized, double-blinded, placebo-controlled | Safety and efficacy | 2 i.m. doses | 210 patients (18–50 yr) | June, 2022 Genexine, Inc. NCT04445389 |
| COVID-19/aAPC | 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. | Open-label trial | Safety and immune reactivity | 1 s.c. dose | 100 patients (60–80 yr) | December, 2023 Shenzhen Geneimmune Medical Institute NCT04276896 |
| APC, antigen-presenting cell. | 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. | Open-label trial | Safety and immune reactivity | 3 s.c. doses | 100 patients (60–80 yr) | December, 2024 Shenzhen Geneimmune Medical Institute NCT04276896 |

APC, antigen-presenting cell.

**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 evolution 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 above-mentioned 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 Peruana Cayetano Heredia | NCT04612972 |
| ChAdOx1nCoV-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 | Novavax | 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 |
 Anti-COVID-19 Vaccines

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, paroviruses (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 administered vaccinations [63]. The majority of vaccinations are administered intramuscularly. However, the intradermal administration (NCT03305341 and NCT04477781), oral administration (NCT04349880), 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, NCT04412467, 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, 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 live 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.

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