

## REVIEW

# Interpretative immune targets and contemporary position for vaccine development against SARS-CoV-2: A systematic review

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**Funding information**

Science and Engineering Research Board, Grant/Award Number: EMR/2016/007564; Department Of Science & Technology, India, Grant/Award Number: TDP/BDTD/33/2019; Biotechnology Industry Research Assistance Council, Grant/Award Number: BT/IIPME0211/02/16

**Abstract**

The year 2020 started with the emergence of novel coronavirus, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which causes COVID-19 infection. Soon after the first evidence was reported in Wuhan, China, the World Health Organization declared global public health emergency and imminent need to understand the pathogenicity of the virus was required in limited time. Once the genome sequence of the virus was delineated, scientists across the world started working on the development of vaccines. Although, some laboratories have been using previously developed vaccine platforms from severe acute respiratory syndrome coronavirus (SARS) and middle east respiratory syndrome-related coronavirus and apply them in COVID-19 vaccines due to genetic similarities between coronaviruses. We have conducted a literature review to assess the background and current status of COVID-19 vaccines. The worldwide implementation and strategies for COVID-19 vaccine development are summarized from studies reported in years 2015–2020. While discussing the vaccine candidates, we have also explained interpretative immune responses of SARS-CoV-2 infection. There are several vaccine candidates at preclinical and clinical stages; however, only 42 vaccines are under clinical trials. Therefore, more industry collaborations and financial supports to COVID-19 studies are needed for mass-scale vaccine development. To develop effective vaccine platforms against SARS-CoV-2, the genetic resemblance with other coronaviruses are being evaluated which may further promote fast-track trials on previously developed SARS-CoV vaccines.

**KEYWORDS**

Antiviral agents, Coronavirus, Immunopathology, Immunomodulators, pandemic, Respiratory tract

## 1 | INTRODUCTION

With the emergence of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) outbreak, after the first case was reported at Wuhan China in late December 2019, a rapid transmission to 210 other

countries was emanated causing a global public health emergency on January 30, 2020, declared by the World Health Organization (WHO).<sup>1</sup> Subsequently, in the first emergency meeting of WHO, an estimated 4% fatality rate,<sup>2</sup> 2.2% of reproductive rate, and an incubation period of 5.8–14 days of COVID-19 were noticed.<sup>3</sup> To date, 36.4 million active

cases and more than 1.06 million deaths due to COVID-19 are reported in more than 210 countries and territories.<sup>4</sup> The collaborative efforts of WHO and scientist's community across various countries are evaluating the pathogenesis of COVID-19 and their signaling pathways to target biomarkers for controlling, preventing, and developing new therapeutics against SARS-CoV-2 infection.

The SARS-CoV-2 is positive-sense single-strand RNA (26–32 kb) coronavirus belonging to *Coronaviridae* family, *Coronavirinae* subfamily, and *Nidovirales* order.<sup>5,6</sup> On the basis of their genetic structure, the coronavirus have been classified into alpha ( $\alpha$ ), beta ( $\beta$ ), gamma ( $\gamma$ ), and delta ( $\delta$ ) genera. Among these genera, only  $\alpha$ - and  $\beta$ - coronavirus infect mammals.<sup>7</sup> By evaluating the complete genome sequence of SARS-CoV-2 coronavirus, it was reported that the virus poses a genetic similarity of 96.2% with bat CoV RaTG13 and 70% with SARS-CoV.<sup>7–9</sup> Furthermore, similar to other coronaviruses, SARS-CoV-2 also encodes structural proteins, namely nucleocapsid protein (N), envelope protein (E), membrane protein (M), and spike protein (S).<sup>7</sup> The immunopathogenicity of SARS-CoV-2 coronavirus was studied on the basis of genetic similarity with SARS-CoV causing protective immune responses against SARS-CoV-2.<sup>7,10–12</sup> These studies facilitate the understanding of immune responses against SARS-CoV-2 which are potentially leveraged for vaccine development. Epidemiological, clinical, laboratory, and radiological outcomes of confirmed 2019–Novel coronavirus (2019-nCoV) infection in patients were analyzed by Huang et al.<sup>13</sup> and the symptomatic similarity among hospitalized and nonhospitalized COVID-19 patients were confirmed. In this study, ICU patients demonstrated higher plasma level of proinflammatory cytokines, namely IL2, IL7, IL10, GSCF, IP10, MCP1, MIP1A, and TNF- $\alpha$ . Moreover, humoral immune response in 208 clinical subjects against recombinant viral N-proteins was evaluated confirming the generation of detectable IgM and IgA within 5 days and IgG within 14 days of infection.<sup>14</sup> The detection efficiency of IgM-Enzyme-Linked Immunosorbent Assay (IgM-ELISA) was found higher in comparison to quantitative Polymerase Chain Reaction (qPCR); however, combining IgM-ELISA with qPCR significantly improved the positive detection rate (98.6%). Similar to SARS-CoV and middle east respiratory syndrome-related coronavirus (MERS) coronaviruses, antibody response in COVID-19 patients was also short-termed as compare to T-cell response, therefore, developing an effective epitope-based vaccine against SARS-CoV-2 infection was recommended.

Initially, the combination of antiviral drugs, such as chloroquine, remdesivir, favipiravir, and arbidol, along with antibiotics were prescribed for SARS-CoV-2 infection in clinical patients.<sup>15–17</sup> Randomized clinical trials were studied by administering lopinavir-ritonavir (1:1 ratio) in COVID-19 patients twice a day for 14 days. A similar mortality rate between treated and standard care groups was reported.<sup>18</sup> Severe gastrointestinal adverse effects in the standard care group were noticed, and therefore, treatment was stopped in 13.8% patients. Furthermore, irregular heartbeats and cardiac arrest in patients prescribed with hydroxychloroquine (HCQ) and antibiotic azithromycin against SARS-CoV-2 pneumonia were also reported.<sup>19,20</sup> Therefore, HCQ was approved by the food and drug administration (FDA) on March 30, 2020, for only hospitalized suspected or confirmed COVID-19 patients under emergency use authorization.<sup>21</sup> On

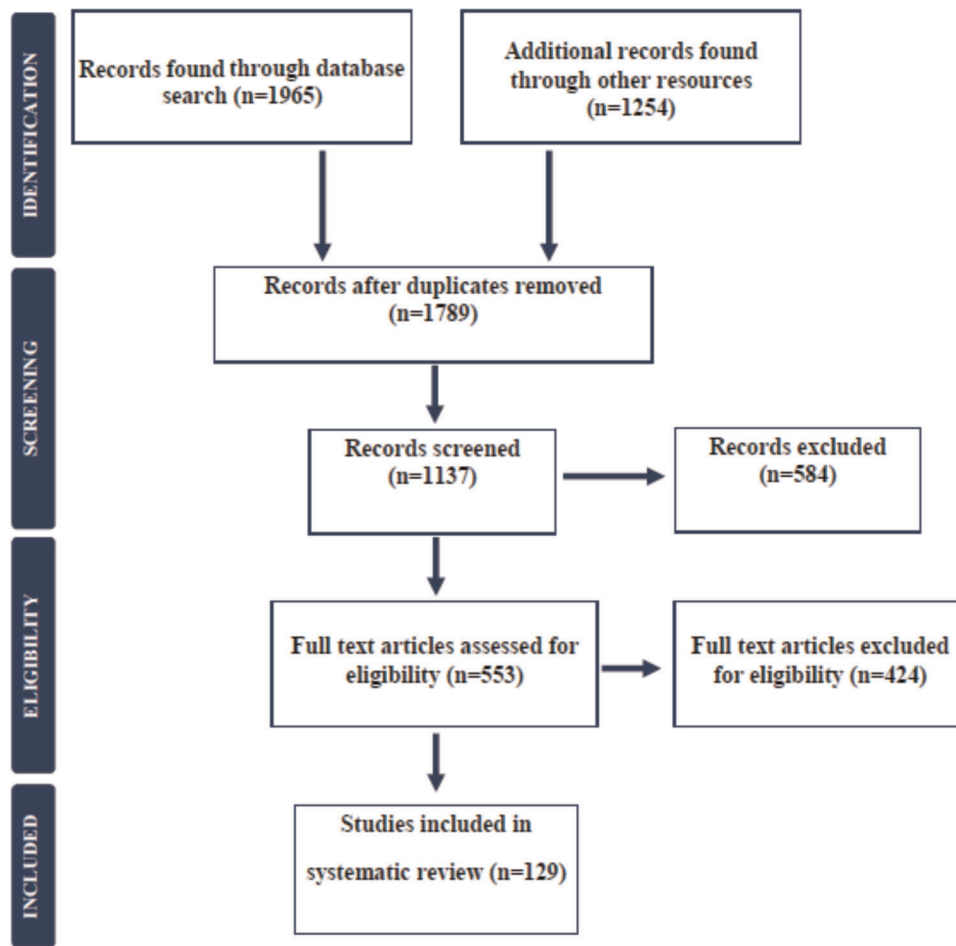
the other hand, convalescent plasma or immunoglobulins based treatment therapy was also provided to COVID-19 patients. It has been previously prescribed in H1N1, HIV-1, and Ebola viral infections.<sup>22</sup> However, plasma therapy is not efficient in end-stage COVID-19 patients.<sup>23</sup> In considering forgoing passive antibodies, a short period of time and their regulatory and logistical challenges limit their use in the current situation.<sup>24</sup> Contemplating various challenges associated with treatment therapies against SARS-CoV-2 coronavirus, while investigating cross-resistance of other vaccines with COVID-19, the new candidates for vaccines have been evaluated.<sup>25–28</sup> Presently, over a dozen potential candidates including recombinant protein subunits, oligonucleotides, and live viruses are being evaluated against SARS-CoV-2. Besides, more than 100 preclinical studies, 42 clinical trials are being analyzed to develop new and effective vaccine therapy.<sup>29</sup> However, formal testing toxicology study, additional manufacturing steps, and finding volunteers to study COVID-19 specific vaccine are continuously delaying the development. The funds from manufacturers who were previously developing vaccines for SARS and Zika viruses were reallocated by federal funding agencies since the pandemic associated with these viruses were ended before vaccine development was completed.<sup>25</sup> Therefore, to understand the accurate pathogenesis and discovery of potential targets for effective therapeutic intervention, extensive and rapid research is required for vaccine development against SARS-CoV-2 coronavirus. In this systematic review, the immune responses against SARS-CoV-2 coronavirus and their potential targets for vaccine development are covered. Furthermore, emerging vaccine development technologies and the current status of major vaccines in clinical and preclinical studies for COVID-19 are discussed.

## 2 | MATERIALS AND METHODS

To investigate the relevant studies on vaccine development for COVID-19, a literature search for the last 5 years was conducted in databases including PubMed, Google Scholar, and online web resources. The search keywords were SARS-CoV-2, coronavirus, NCoV, 2019-CoV, COVID-19, 2019-nCoV, etiology, pathogenicity, immunogenicity, humoral- and cell-mediated immune signaling, antibodies, drug treatment, antiviral drugs, immunotherapy, vaccine, vaccine market, preclinical study, and clinical trials. However, unpublished data, thesis work, articles published in languages other than English, and press releases were excluded. Furthermore, to provide additional relevant information, review articles evaluating vaccines against COVID-19 treatment were profoundly studied.

## 3 | RESULTS

This review assimilates the search results of 1789 articles after preliminary screening conducted on PubMed, Google scholar, and web resources. Abstract and methodology screening were then performed and 1137 articles and documents were shortlisted. The



**FIGURE 1** Flow diagram to illustrate selection criteria for the systematic review on COVID-19 vaccines

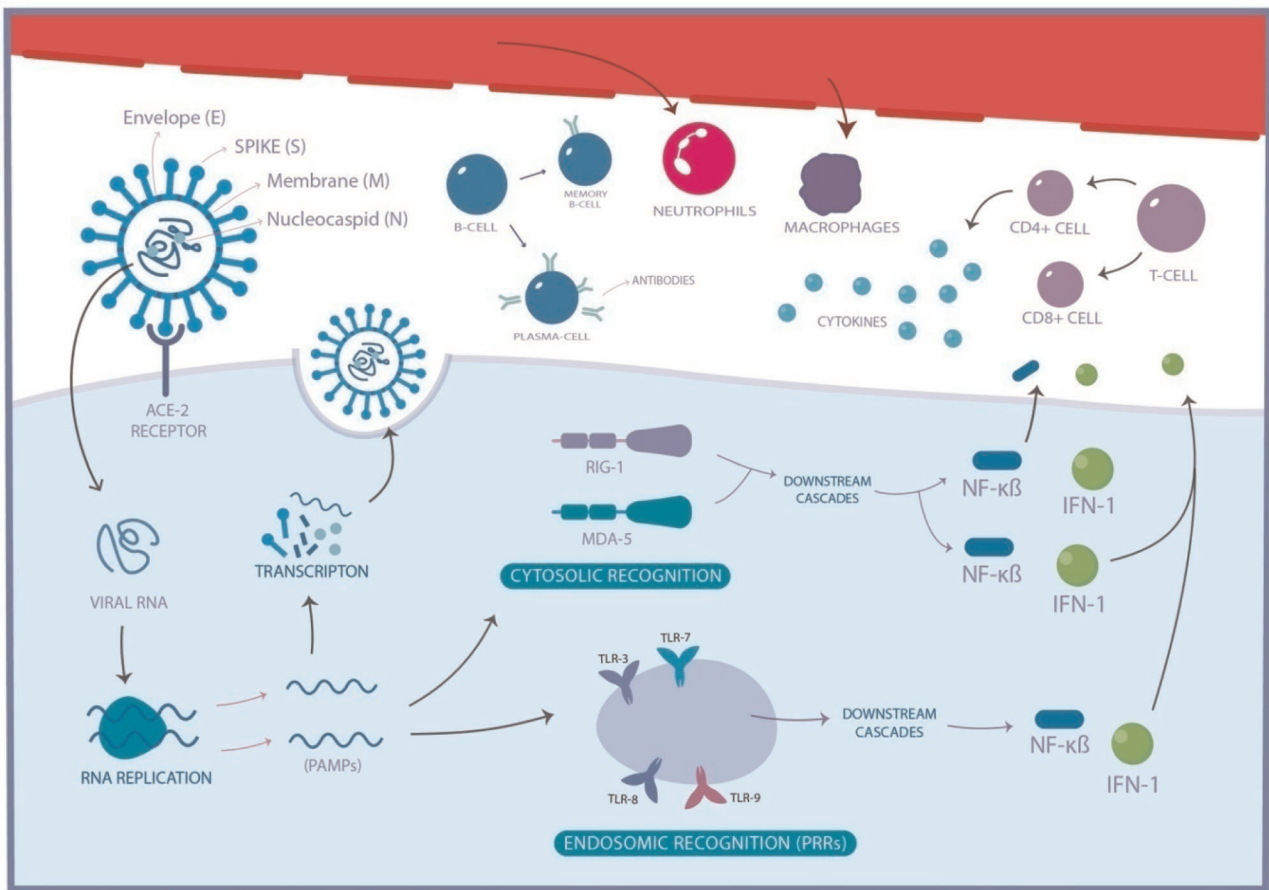
flowchart of the final articles selection is illustrated in Figure 1. Furthermore, 424 articles and documents were excluded after the full-text screening, among them 49 articles were also in languages, other than English and 189 articles did not specify relevant results for a vaccine against SARS-CoV-2 coronavirus.

## 4 | DISCUSSION

First step toward vaccine development for COVID-19 is to understand the mechanism and functionality of immune response against SARS-CoV-2 coronavirus. However, there is no cellular-based study on evaluating the complete cycle of COVID-19 induced immune response in an infected person is performed. Although, considering genetic and proteomics similarity of SARS-CoV-2 with other coronaviruses including SARS-CoV and MERS, the potential immune signaling pathways against SARS-CoV-2 can be predicted,<sup>7,10–12</sup> as shown in Figure 2. On the basis of numerous studies, the possible potential immune responses in COVID-19 patients are determined.

During the process of SARS-CoV-2 infection, proteolytic processing of S-protein is occurred once host cell receptor protein angiotensin-converting enzyme 2 binds with C-terminus of S1-

subunit of S-protein resulting in a protease activity which allows fusion of the virus to the host cell membrane.<sup>12,30–32</sup> Most viral RNA act as pathogen-associated molecular patterns and detected by pattern recognition receptors, namely toll-like receptor (TLR) types 3, 7, 8, and 9 in the endosome.<sup>12,14,33</sup> Furthermore, RNA receptor retinoic-acid inducible gene I, cytosolic receptor melanoma differentiation-associated gene 5, and nucleotidyltransferase cyclic GMP-AMP synthase facilitate the cytosolic recognition of the virus. These complex signaling triggers downstream cascade molecules and activates NF- $\kappa$ B, IFN- $\alpha/\beta$ , and few proinflammatory cytokines.<sup>12,14,34</sup> Cytokines storm and dysregulated immune responses further induce respiratory disease pathogenesis caused by SARS-CoV-2 which is similar to SARS-CoV and MERS. In addition, the imbalances in levels of interleukins, GCSF, macrophages, colony-stimulating factor, interferons, and the complement system are reported in patients suffering from COVID-19.<sup>8,13,35,36</sup> Large number of SARS-CoV-2 specific CD4<sup>+</sup> and CD8<sup>+</sup> T-cells subtypes were reported in COVID-19 convalescent patients.<sup>37–39</sup> However to envisage a larger perspective, cohort studies are needed to perform to examine whether the excess of T cells are protection or conversely associated with the pathogenesis of SARS-CoV-2 coronavirus. Therefore, these studies in understanding mechanistic



**FIGURE 2** Potential immune system response against SARS-CoV-2 coronavirus infection

insights of COVID-19 pathogenesis, the outcome attributed to new vaccine intervention will be promising.

#### 4.1 | Potential targets of vaccine development for COVID-19

The vaccines are immune boosters against invading viruses by recognizing unique molecular candidates found on their surface or in the genome. Ideally, the human immune system responds against viral antigens by secreting proteins or special immune cells that either directly attacks the pathogen or indirectly via inducing other immune cells to destroy the virus. Therefore, knowledge of viral target is very much required in vaccine development to improve the immunogenicity and prevention against COVID-19. However, scientists are focusing on available data of SARS-CoV-2 immunogenicity and genetic similarity of coronaviruses to develop associated vaccines.

Studies on immune responses against SARS-CoV-2 epitopes, viral nucleotide, viral structural and nonstructural proteins, which may act as a target in vaccine development, are expeditiously effectuated.<sup>15,40–42</sup> On the basis of genetic and proteomics similarities between SARS-CoV and SARS-CoV-2, potential immune targets

for COVID-19 vaccine development are being assessed in various research institutions and industrial R&D laboratories in numerous countries. The antibody-based neutralization has been a major target for traditional vaccines since receptor-mediated cell-binding assists in modulating pathogenicity by administering specific toxins and thereby promoting protective antibody production. Newer vaccines for complex pathogens are designed to target antibodies, CD4<sup>+</sup>, and CD8<sup>+</sup> T cells as immunotherapy of persistent or recurrent infections. Structural and nuclear proteins of SARS-CoV-2 coronavirus along with receptor-binding domain (RBD) and N terminal domain of S-protein are targeted for currently developing vaccines.<sup>42,43</sup> Several studies are being performed using chemical and immunoinformatic approaches to design epitope vaccine against COVID-19. Immunoinformatic tools to identify SARS-CoV-2 specific S- and N-proteins derived B- and T-cell epitopes which could help in developing immunogen to prevent the COVID-19 was developed.<sup>11</sup> Homogeneity of 23% and 16% of T-cell and B-cell epitopes between SARS-CoV and SARS-CoV-2 coronaviruses were identified respectively which may further facilitate our studies on the development of a new vaccine against SARS-CoV coronavirus. In addition, immunoinformatic strategies on B- and T-cell epitopes demonstrating binding to antigenic human leukocyte antigen alleles and inducing immune responses were performed.<sup>44–47</sup> B-cell epitopes ( $n = 34$ ) and

MHC-I and MHC-II bounded T-cell epitopes<sup>44</sup> (29 and 8 epitopes, respectively) were analyzed which can be the target for vaccine development against COVID-19. Thus, genetic similarities in epitopes among SARS family coronaviruses can promote next-generation vaccine development that will target future pandemic of other coronaviruses as well.

## 4.2 | Current status of vaccine development for COVID-19

Once identifying the genetic sequences of SARS-CoV-2 coronavirus, effective vaccine development against the disease has been prioritized since March, 2020.<sup>26</sup> As per Coalition for Epidemic Preparedness Innovations database, total of 180 vaccine candidates were selected against COVID-19 by September 2020, among them only 42 vaccines are under clinical trials.<sup>29</sup> More fast-track processes for vaccine development are encouraged which can rapidly promote the commercialization of vaccine candidates.

### 4.2.1 | Nucleic acid vaccine

The nucleic acid vaccines are determined by the functioning of inserted nucleic acid into viral or nonviral vectors and thereby stimulate immune response against pathogen.<sup>40,48–50</sup> These vaccines are safe and still have significant therapeutic potential without the use of adjuvants. Nucleic acid vaccines are able to induce both B and T-cell responses and targeted immunogenicity and can easily be implemented in generic manufacturing processes for effective and rapid responses.

#### *Nanocarrier based nucleic acid vaccine*

At the initial stage, the National Institute of Allergy and Infectious Diseases (NIAID) and Moderna Inc., were the first to get FDA approval to use mRNA vaccine (mRNA-1273) in fast track designation and completed clinical trial phase II (NCT04405076). This mRNA-1273 is lipid nanoparticle (LNP)-encapsulated mRNA-based vaccine that encodes SARS-CoV-2 S-proteins and recently entered in phase III clinical trial (NCT04470427). Two doses of this vaccine reported enhanced neutralization activity and binding of IgG geometric mean titers (GMTs) against S2-P and CD4 T-cell responses against Th1 cytokines in healthy participants.<sup>51</sup> Furthermore, BioNTech SE and Pfizer Inc. have collectively developed four candidates of LNP-encapsulated mRNA vaccine named BNT162 against COVID-19. The LNP covered vaccine candidates have two nucleosides modified and one single uridine containing and one self-amplifying mRNA vaccine candidates; among them, two vaccine candidates have included larger spike sequences and the other two have smaller RBD domain of S-protein. The two vaccine candidates, namely BNT162b1 and BNT162b2, from this multistage study have been registered for III clinical trial later in April 2020 (NCT04368728) against COVID-19 infection.

Another LNP encapsulated self-amplifying ribonucleic acid (saRNA) vaccine for COVID-19, namely nCoVsaRNA is developed by Imperial College of London and is recently undergoing in phase I clinical trial (ISRCTN17072692). The manufacturing license of saRNA vaccine is transferred to TriLink BioTechnologies, San Diego, for large scale vaccine production for upcoming clinical trials. In addition, the CVnCoV COVID-19 LNP based mRNA vaccine produced by CureVac AG, Germany, has completed phase I clinical trial and recently entered in phase II clinical trial (NCT04515147) to evaluate effective vaccine multi-dosage along with Pneumococcal and Hepatitis A vaccines as control. In continuation, nanoparticle-based nonviral delivery system, LUNAR<sup>®</sup> based self-replicating RNA was combined with STARR<sup>™</sup> (Self-Transcribing And Replicating RNA) Technology platform and adopted by Arcturus Therapeutics Holdings Inc., San Diego and Duke-NUS Medical School (Duke-NUS), Singapore to develop COVID-19 vaccine candidate. This vaccine was named ARCT-021 and recently entered in phase I/II clinical trial (NCT04480957).

The catatonic outer membranes of LNPs have reported previously and have advantages over conventional delivery system including targeted drug delivery, improved biocompatibility, less adverse events, and protection of oligonucleotide-based therapeutics from cellular metabolism.<sup>52–54</sup>

#### *Other nucleic acid-based vaccines*

Recently, a DNA vaccine candidate INO4800 which is designed by Inovio Pharmaceuticals, Pennsylvania, can be delivered to human cells and induce an immune response against SARS-CoV-2 coronavirus. It is administered intradermally by CELLECTRA 2000 electroporation method (NCT04336410) and is still in phase I clinical trial. Electroporation technique can cause higher pain and discomfort in comparison to the conventional vaccine delivery methods at the site of injection. Two DNA vaccines, GX-19 (Genexine Inc., South Korea) and ZyCov-D (Cadila Healthcare Ltd., Ahmadabad, India), and one mRNA vaccine, ARCoV (People's Liberation Army [PLA] Academy of Military Sciences and Walvax Biotech Co. Ltd., China) are designed recently. These vaccines against SARS-CoV-2 infection have entered in clinical trial, NCT04445389 (phase I), CTRI/2020/07/026352 (phase I/II), and ChiCTR2000034112 (phase I), respectively. Another attempt is made by Takara Bio Inc., Japan, together with Osaka University and AnGes, Inc. Japan group to prepare the AG0301-COVID19 vaccine. This vaccine is combined with adjuvant and entered in phase I/II clinical trial (NCT04463472).

#### *Preclinical studies on nucleic acid vaccine against SARS-CoV-2*

In addition to these clinical trials, 17 and 12 preclinical studies on RNA and DNA SARS-CoV-2 vaccines are still undergoing across various countries. The LNP-based non-viral vector exhibits enhanced antigen expression and easy cellular uptake along with protecting mRNA from degradation.<sup>55,56</sup> Therefore, CanSino Biologics Inc. and Precision NanoSystems, Fudan University, Shanghai, JiaoTong University, Shanghai and RNACure Biopharma, and University of Tokyo and Daiichi-Sankyo companies have



started developing LNP encapsulated mRNA vaccine candidates targeted against COVID-19 infection.

#### 4.2.2 | Non-replicating viral vector vaccine

For developing a vaccine against COVID-19 infection, a non-replicating viral vector technology that is encoded by the process of antigen delivery through an unrelated vector shuttle is developed. This is a promising approach to induce immune response against the antigen non-replicating property of the vector. This process allows the vector to reproduce multiple copies causing vector to infect single cell, transmitting viral immunogens without causing viral infection.<sup>57-62</sup> WHO has reported 20 non-replicating viral vector platforms for COVID-19 vaccine development with eight vectors are progressive toward clinical trials.<sup>29</sup>

##### *Adenovirus type 5 vector-based vaccines*

CanSino Biological Inc., China, in collaboration with the Beijing Institute of Biotechnology has developed non-replicating adenovirus type 5 (Ad5) vector-based vaccine against COVID-19. The studies of these clinical trials (NCT04313127) are reported by analyzing the safety and tolerability of vaccine in healthy volunteers.<sup>63</sup> The healthy subjects were administrated with  $5 \times 10^{10}$  viral particles per 0.5 ml,  $1 \times 10^{11}$  viral particles per ml, and  $1.5 \times 10^{11}$  viral particles per 1.5 ml vaccine dose in phase I clinical trial. The outcomes illustrated that high-dose subjects reported adverse events including muscle or joint pain, fatigue, and high fever, whereas low doses of vaccine boosted neutralizing and anti-RBD antibodies in test subjects. Therefore, low and middle vaccine dose is selected for phase II clinical trial to target full-length S-protein of SARS-CoV-2 coronavirus (NCT04341389) and reported  $5 \times 10^{10}$  viral particles as safe dose along with high immune responses in 508 health volunteers.<sup>64</sup>

Another non-replicating Ad5 vector-based vaccine is developed by the University of Oxford in collaboration with AstraZeneca Plc, Cambridge, and has further licensed to Serum Institute of India, Pune, for large scale manufacturing of AZD1222 (ChAdOx1) vaccine. The ChAdOx1 is a chimpanzee-based adenovirus vector vaccine that stimulated both humoral and cytotoxic T-cell responses with respect to SARS-CoV-2 S-protein in animal models.<sup>65</sup> The clinical trial study of the developed vaccine reported positive outcomes against SARS-CoV-2 infection.<sup>66</sup> The single dose of  $5 \times 10^{10}$  viral particles generated antibodies against S-protein in ChAdOx1 nCoV group and gradually increased from day 28 to 56. Though, the proposed vaccine has completed phase III clinical trial (ISRCTN89951424) and paused in several countries due to neurological complications in volunteers.

Recently, Russia based Gamaleya Research Institute has identified another adeno-based vaccine against COVID-19 infection and completed the phase I clinical trial (NCT04436471; NCT04437875). These vaccines, Gam-COVID-Vac Lyo and Gam-COVID-Vac (recombinant Ad26 [rAd26] and recombinant Ad5 [rAd5]) are present in lyophilized and frozen formulations respectively and can be administered intramuscularly. Phase I/II study of the above vaccines

demonstrated increased immune responses, yet rAd26 did not neutralize proteins adeno virus specific proteins, therefore, it was used for priming, and rAd26-S was used for boosting after 28 days. This is an excellent method to overcome the immune response generated against components of the viral vector. These vaccines have been already registered by the Government of the Russian Federation (registration no: LP-006395 [Gam-COVID-Vac] and LP-006423 [Gam-COVID-Vac-Lyo]) without publishing phase III clinical trial (NCT04530396) results of the vaccines.

Vaxart Inc., USA has also initiated a new program, namely VAAST oral vaccine platform in which vaccine may induce immunization in SARS-CoV-2 patients, though this vaccine is still in clinical trial phase I (NCT04563702). In another attempt, Janssen Pharmaceutical used Janssen AdVac technology to develop the Ad26COVS1, non-replicating Ad5 vector vaccine against SARS-CoV-2 coronavirus. This vaccine candidate has already demonstrated an enhanced immune response by neutralizing antibodies based infection diminishing in non-human primate SARS-CoV-2 virus model.<sup>67</sup> Similarly, the Academy of Military Medical Sciences, PLA of China has also designed Ad5 vector-based non-replicating vaccine candidate, namely Ad5-nCoV, and currently under phase I clinical trial (NCT04552366). The detailed information on this vaccine is not available to date. The Ad5 vector vaccine does not integrate with the host genome after entering the nucleus,<sup>68</sup> thus avoids risk of insertional mutagenesis and is safe to use in human. The quiescent and actively dividing cells were transduced to give high titers and thereby allowing high protein expression. These advantages make the Ad5 vector an efficient target as vaccine candidate vector for COVID-19 treatment. However, ReiThera Srl, Italy has designed replication-defective gorilla adenoviral (GRAd) vector-based COVID-19 vaccine, namely GRAd-CoV2. This vector is closely related to human species C adenovirus virus, thus it can be highly significant in SARS-CoV-2 infection prevention. In another study, Ludwig-Maximilians-University, Munich, Germany designed a modified vaccinia virus Ankara (MVA) based non-replicating viral vector vaccine against SARS-CoV-2 and under clinical trial phase I (NCT04569383).

##### *Preclinical studies on non-replicating viral vector vaccines*

Besides, 18 preclinical studies from various companies and organizations to develop non-replicating viral vector-based vaccine against COVID-19 are currently going on namely GeoVax Inc. and BravoVax Co. Ltd.; Altimmune; Greffex Inc.; Stabilitech Biopharma Ltd.; Valo Therapeutics Ltd.; Vaxart Inc.; and research institutions such as DZIF-German Center for Infection Research, Germany; Centro Nacional Biotecnología (CNB-CSIC), Spain; University of Manitoba, Manitoba; the University of Georgia and the University of Iowa; Bharat Biotech International Ltd., Hyderabad; Thomas Jefferson University, Pennsylvania.<sup>29</sup> Among them, GeoVax Inc. and BravoVax Co. Ltd.; DZIF, and CNB-CSIC use MVA vector, whereas Janssen Companies, Altimmune, Greffex Inc., Stabilitech Biopharma Ltd., and Valo Therapeutics Ltd. use Ad5 vector to report SARS-CoV-2 specific S-protein. Considering their preliminary success, non-replicating viral vector vaccines may have considered as safe and highly immunogenic against SARS-CoV-2 infection.

### 4.2.3 | Replicating viral vector vaccine

The replicating viral vector vaccine exploits the potential of vectors to continuously replicate and facilitate persistent immune response in the host cell.<sup>68–70</sup> Because of the properties of persistent responses through replicating viral vector vaccine, as compared with non-replicating viral vector vaccine, the viral vector vaccines promote longer and higher expression of immune cells even at lower immunization doses.<sup>69–71</sup> Though, similar to live attenuated vaccine, replicating viral vector vaccine can also revert to their virulence state. Therefore, utilization of replicating viral vector vaccine is still a major concern over regulatory guidelines considering their potential risks and kind of possible safety issues associated with replicating viral vector vaccine.

#### *Clinical trials on replicating viral vector vaccines*

To date, only two vaccine candidates are designed on the concept of replicating viral vectors. One of them is measles virus (MV) vector-based vaccine, namely TMV-083 by Institut Pasteur, Paris in collaboration with Themis, Australia, and University of Pittsburgh, Pennsylvania. This vaccine recently entered in phase I clinical trial (NCT04497298) and safety and immunogenicity are under evaluation in health volunteers. On another hand, collaborative effort of Beijing Wantai Biological Pharmacy Enterprise Co. Ltd. and Xiamen University, China developed an intranasal flu vector-based vaccine for COVID-19 therapy. This vaccine, namely DelNS1-2019-nCoV-RBD-OPT1 recently entered in phase I clinical trial (ChiCTR2000037782) to prove their safety and efficacy in healthy volunteers.

#### *Preclinical studies on replicating viral vector vaccines*

To develop COVID-19 vaccines, 17 replicating viral vector vaccines are under preclinical trials to date.<sup>29</sup> The MV vector vaccines are reported to stimulate memory B-cells and T-cells to induce both humoral and cellular mediated immune responses and provide long-term immunity against viral pathogens.<sup>72</sup> Similar to Ad5, MV vector vaccine also replicates in the cytoplasm, therefore, no integration between viral and host genome occurs and vector may be safe to use in lethal diseases, such as COVID-19. Reverse genetics is used to prepare rMV vector by Zydus Cadila Ltd., Ahmedabad, India and has incorporated codon-optimized protein to stimulate long-term generation of neutralizing antibodies. Depending upon the success in stimulating long-lasting immune response in preclinical studies, the vaccine may enter in clinical phase I by the end of the year 2020.

In collaboration with the University of Wisconsin–Madison, Wisconsin with FluGen Inc. and Bharat Biotech International Ltd., CoroFlu live vaccine is currently under evaluation in preclinical model. The CoroFlu is similar to the influenza vaccine M2-deficient single replication which is FluGen's flu vaccine candidate and administered intranasally to boost immune response against SARS-CoV-2 coronavirus. Moreover, various research and development on a wide range of antigenic genome insertion and strong immune response of influenza virus vector-based vaccines for

COVID-19 prevention are carried out. The attenuated influenza virus backbone based live viral vectored vaccine (BiOCAD Global, Russia and I.E.M., Malaysia), Influenza A virus-based recombinant vaccine (FBRI SRC VB VECTOR, Rospotrebnadzor), attenuated Influenza vector expressing S-protein (Fundação Oswaldo Cruz and Instituto Buntantan, Brazil) and Influenza vector vaccine expressing RBD (University of Hong Kong [HKU]) are presently in preclinical study against SARS-CoV-2. The vaccine developed by HKU is developed on the concept of flu-based DelNS1 live attenuated influenza virus platform, in which SARS-CoV-2 RBD domain-containing flu-vector may stimulate the immune system against COVID-19.

Vesicular stomatitis virus (VSV) vector-based vaccines are separately developed by International AIDS Vaccine Initiative (IAVI) with Batavia Biosciences and University of Western Ontario and FBRI SRC VB VECTOR, Rospotrebnadzor.<sup>29</sup> The preclinical studies of replicating VSV chimeric virus technology presenting SARS-CoV-2 S-protein against COVID-19 prevention are performed by IAVI and Batavia Biosciences.

### 4.2.4 | Inactivated vaccine

The inactivated vaccine consists of virus-like particles that have lost their pathogenicity but are still able to stimulate the human immune response.<sup>73–75</sup> The major advantage of the inactivated vaccines is their ability of retention to non-pathogenic phenotype after inactivation. Currently, 15 inactivated vaccines are under process against SARS-CoV-2, among them only 6 inactivated vaccines are under clinical trials.<sup>29</sup>

The China National Pharmaceutical Group, Sinopharm, have produced two inactivated vaccines in collaboration with Wuhan Institute of Biological Products and CNBG-Beijing Institute of Biological Products Co. Ltd. Both inactivated vaccines, namely sIPV and DTaP have completed phase I/II after approval from Chinese Medical Authorities and recently entered in phase IV clinical trial. The first vaccine was developed by  $\beta$ -propiolactone induced inactivation of coronavirus cells and incubated with alum adjuvant against SARS-CoV-2 infection. The phase I/II study demonstrated significant-high GMT of neutralizing antibodies and TH2 cell response against the virus at very low dose.<sup>76</sup> They have recently entered in phase IV clinical trial (NCT04053010) to evaluate the protective efficacy of the designed vaccine after full course of immunization. Besides, an inactivated vaccine is developed after studying safety and immunogenicity under phase I clinical trial (NCT04412538) by the Institute of Medical Biology, Chinese Academy of Medical Sciences. The collaboration of Sinovac Research and Development Co. Ltd., China, with several laboratories within China have demonstrated positive responses of PiCoVacc, an inactivated vaccine in a combination of adjuvant on an animal model for COVID-19.<sup>77</sup> The PiCoVacc has shown to generate RBD- and N-protein based specific antibodies and thereby inducing an immune response in mice and S-specific IgG and consequently neutralize antibodies in COVID-19 animal model rhesus macaques. These immune responses may

be indicative for developing vaccines for humans, therefore, currently the effect of vaccine completed phase I/II clinical trials (NCT04383574; NCT04352608) and entered in phase III clinical trial (NCT04456595). An India-based industry, Bharat Biotech has also developed a whole-virion inactivated vaccine, BBV152 (COVAXIN), and approved for phase I/II clinical trial (NCT04471519). The QAZCOVID-IN is another inactivated COVID-19 vaccine developed by the Research Institute for Biological Safety Problems, Republic of Kazakhstan in August 2020. The detail of this vaccine is still unavailable yet it has entered phase I/II clinical trial (NCT04530357).

A collaboration of Angenics Inc. and Osaka University, Japan leads to the design and development of target binding domain-based inactivated vaccine against COVID-19. The vaccine is under preclinical study however Angenics Inc. expects to launch the vaccine in a clinical trial by later in the year 2020. Other preclinical studies on inactivated virus along with adjuvant, CpG 1018 are undergoing development by Dynavax Technologies Corporation, California in collaboration with Valneva SE, France, and Sinovac Biotech Ltd., China. The CpG 1018 is TLR9 agonist to induce T<sub>H</sub> cells resulting in immune response and have previously demonstrated positive effects on hepatitis B patients,<sup>78</sup> thus, it could be a potential target for the TLR9 receptors in COVID-19 patients and can be utilized as preventive measurements.

#### 4.2.5 | Protein subunit vaccine

Protein subunit vaccines stimulate human immune response while presenting viral proteins, as a whole or otherwise, without any introducing viral vector.<sup>79,80</sup> Similar to inactivated vaccines, subunit vaccines are also safe, do not risk for disease revert, however, good adjuvant need to be added for high immunogenicity.<sup>79,81,82</sup>

##### *Protein subunit and adjuvant based vaccine*

A protein subunit vaccine NVX-CoV2373 developed by Novavax Inc., USA, is in clinical trial phase III (2020-004123-16). This subunit vaccine is designed on the basis of a full-length recombinant SARS-CoV-2 S-protein nanoparticle along with Matrix M adjuvant. The Matrix M adjuvant has previously reported for generating high antibodies-based positive immune response in H5N1<sup>83</sup> and H7N9 (NCT02078674) pathogens, thus it could be a potential adjuvant mediator in SARS-CoV-2 therapeutic intervention. The COVAX is an Advax-CpG55.2 adjuvant platform-based vaccine for COVID-19, which is developed by Vaxine Pty Ltd., Adelaide, and Medytox Inc., Korea. The immunogenic response of this vaccine is under evaluation and recently entered in phase I clinical trial (NCT04453852). The Advax is delta inulin, a high-temperature tolerant polysaccharide isoform that has previously reported in inactivated Japanese Encephalitis virus and influenza vaccines.<sup>84</sup>

An innovative approach, Trimer-Tag technology-based SARS-CoV-2 S-protein subunit-trimer vaccine (S-trimer), SCB 19 has been developed by Clover Biopharmaceuticals, USA, in collaboration with Dynavax Technologies Corp., USA. After studying the effect of SCB

19 on COVID-19 patients along with AS03 and CpG 1018 adjuvants, the vaccine is in phase I clinical trial (NCT04405908). Similarly, the collaborative effort of Medigen Vaccine Biologics Corp., Taiwan, and Dynavax Technologies Corp., USA, resulted in the development of another protein subunit vaccine. This vaccine candidate contains SARS-CoV-2 S-protein along with CpG 1018 adjuvant and recently entered in phase I clinical trial to evaluate safety and immunogenicity in healthy volunteers (NCT04487210).

In an attempt to generate S-protein specific antibodies, Kentucky Bioprocessing Inc., USA (NCT04473690) and Anhui Zhifei Longcom Biopharmaceutical Inc., China, along with the Institute of Microbiology, Chinese Academy of Sciences (NCT04466085) have also designed RBD based protein subunit vaccine against COVID-19 and recently entered in clinical trials. Furthermore, the University of Queensland, Australia used a novel approach of the molecular clamp to developed a stabilized S-protein MF59 adjuvant-based vaccine against COVID-19. The research team partner with CSL Ltd. for mass production in phase I clinical trial (1ACTRN12620000674932p). The Instituto Finlay de Vacunas, Cuba has also designed a protein subunit vaccine, namely FINLAY-FR-1 along with adjuvant to target RBD domain of SARS-CoV-2 coronavirus and recently entered in phase I/II clinical trial (IVF/COR/04) to evaluate the safety profile and immune responses. The FBRI SRC VB VECTOR, Rospotrebnadzor is moving forward with peptide subunit vaccine, namely EpiVacCorona against SARS-CoV-2, and recently registered for phase I clinical trial (NCT04527575). Other than DelNS1-2019-nCoV-RBD-OPT1, a replicating vector-based COVID vaccine, almost every vaccine is intramuscularly administered. Therefore, University Hospital Tuebingen designed a protein subunit vaccine, namely CoVac-1, which is subcutaneously administered and recently entered in phase I clinical trial (NCT04546841). Though recently COVAXX, United Biomedical Inc. has developed a unique multipeptide-based vaccine, namely UB-612 which is a genetic fusion of RBD to single-chain Fc domain of IgG1. This promising protein subunit vaccine is still under clinical trial phase I (NCT04545749).

##### *Baculovirus based vaccine*

At first, West China Hospital, Sichuan University developed SARS-CoV-2 RBD protein-specific vaccine candidate in baculovirus based vector. This is the first study to use insect cell culture for vaccine production and got approval for a clinical trial in China (ChiCTR2000037518). The non-pathogenicity and large scale cloning characteristics of baculovirus may show an effective result of the developed vaccine in COVID-19 patients. Similarly, another baculovirus based vaccine candidate is developed by Sanofi S.A., France, and novel adjuvant technology of GlaxoSmithKline Plc., England, and currently is under phase I clinical trial (NCT04537208). Their 2×2 study to evaluate immunogenicity has incorporated two different formulations of vaccines with two adjuvants in healthy young (<18 years) and old volunteers.

##### *Preclinical studies on protein subunit-based COVID-19 vaccines*

The studies on the strong binding of the RBD domain of S-protein and ACE-2 receptor encourage the development of RBD-based



vaccine for COVID-19. In this direction, a Switzerland-based NEOVII Biotech Inc., together with Tel Aviv University, Israel, has performed preclinical study to check the efficacy of RBD based protein subunit vaccine on SARS-CoV-2 coronavirus. The collaborations of Chulalongkorn University and Government Pharmaceutical Organization, Thailand; Biological E Ltd.; Baylor College of Medicine, Texas; NEOVII, Switzerland and Tel Aviv University, Israel; Baiya Phytopharm and Chula Vaccine Research Center, Thailand lead to the design and development of RBD based vaccines against SARS-CoV-2 coronavirus.

EpiVax Inc. in collaboration with Generex Biotechnology Corporation has been investigating unique li-key immune system activation technology where the segment of li-protein acts at the allosteric site on antigen-binding site of MHC-II molecule enhancing the immune response in COVID-19 animal model. Besides, OncoGen Pharma and MIGAL Galilee Research Institute, Israel have been studying efficacy and immunity of long peptide vaccine of synthetic origin for M and S proteins and oral *E. coli*-based protein expression system for S and N proteins of SARS-CoV-2 coronavirus, respectively. In addition, more than 30 vaccines are currently in preclinical studies and positive results are expected for effective clinical trials.

#### 4.2.6 | Live attenuated vaccine

The live attenuated vaccines contain live fragments of virus or pathogen while virulence property is attenuated as compared to live complete pathogen, and thereby stimulating the human immune response.<sup>75,85,86</sup> However, in some cases, the virulence reversion of live attenuated vaccine can cause tissue damage and infection in immunocompromised hosts.<sup>87,88</sup> Only three live attenuated vaccines are under investigation in preclinical studies for COVID-19 infection, none has entered in the clinical trial due to their virulence nature. The very first and effective preclinical study of live attenuated vaccine is performing by Codagenix Inc. and Serum Institute of India, Pune. A codon deoptimized live attenuated vaccine to promote an immune response against SARS-CoV-2 coronavirus is developed. The potential positive results will be declared in the upcoming months. Furthermore, Indian Immunologicals Ltd. and Griffith University, Queensland have been studying codon deoptimized live attenuated vaccine at preclinical stage and have promised long-lasting protection within single-dose administration.

The summaries of developed COVID-19 vaccines with their current status in clinical trials are discussed in Table 1.

## 5 | CLOSURE AND OPINION ON VACCINE DEVELOPMENT STRATEGIES

The unavailability of effective treatment therapy for COVID-19 infection promotes the rapid development of several antiviral drugs which are still under clinical trials for potential outcomes. However,

these trials will not predict the accurate safety and efficacy of the interventions in a short period of time. Therefore, it is inevitable to find accurate target proteins or respective biomarkers for rapid and correct treatment after conducting large scale clinical trials. In the present scenario, structural, or nuclear proteins of viruses are primary targets for COVID-19 treatment. Moreover, similarity among coronavirus family facilitates the identification of new potential targets for therapeutic advancement. The global R&D efforts against SARS-CoV-2 coronavirus are unprecedented in terms of speed and scale; however, continuous and careful monitoring of associated risks and effectiveness of novel technology for vaccine platforms at each step is necessary. Furthermore, COVID-19 specific animal models including ACE-2 transgenic mice, hamsters, or non-human primates are being examined for the efficacy of new vaccine candidates. Besides, maintaining biosafety level-III measures and coordination for sufficient laboratory practices are required for handling live-virus challenges.

Recently, there is a gradual increase in active cases of COVID-19 infection, and phase I clinical trial requires healthy subjects to check the safety of the drugs or vaccine therapy. As compared to large research institutions, small academics and R&D research laboratories may have developed potential vaccines for COVID-19 with positive results at preclinical level. However, due to a lack of large-scale manufacturer or appropriate funding, the therapy might have not entered into clinical phases.

Vaccine development is a long and complex process despite the active participation of the public and private sectors. The efficacy of the vaccine can be evaluated on the basis of disease prevention, pathogen infection prevention, and production of associated immune cells. However, in the case of COVID-19, there is a requirement of developing vaccines, checking their efficacy, and manufacture at a mass-scale in a shorter period of time since COVID-19 cases are increasing very rapidly across the globe. Therefore, managing these conditions to develop an effective vaccine against COVID-19 will be difficult for scientists as well as manufacturers.

The current approach of vaccine development is a stepwise process where manufacturers keep records of toxicology and efficacy of proposed vaccine candidates. These safety measurements are mandatory for authorized agencies such as FDA or WHO to give approval for clinical trials. Though, rapid mutation-induced genetic variability and evolution in RNA virus can be an additional barrier to the under development of COVID-19 vaccines. Hence, the scientific community needs to focus on more research on personalized vaccines on the basis of geographical and genetic similarity.

To date, DNA plasmid-based vaccine, INO4800 (Inovio Pharmaceuticals, Pennsylvania), non-replicating vaccine, inactivated vaccine, PiCoVacc (Sinovac Research and Development Co. Ltd., China), mRNA-1273 vaccine (Moderna Inc., USA and NIAID), and BNT162 (BioNTech SE, Germany; Pfizer Inc., New York; and Fosun Pharmaceutical, China) continuously entering in next steps of COVID-19 clinical trials. Whereas, Gam-COVID-Vac Lyo and Gam-COVID-Vac (Gamaleya Research Institute, Russia) are registered for clinical use and AZD1222 (the University of Oxford and AstraZeneca Plc, Cambridge) is withdrawn over the globe due to neurological

TABLE 1 Current status of vaccines against COVID-19 under clinical trials

SN	Vaccine name	Vaccine platform	No. of doses	Mode of administration	Manufacturer/collaborator	Current status
1.	mRNA-1273	LNP-encapsulated mRNA vaccine targeting whole S-protein	2	Intramuscular	Moderna Inc. and National Institute of Allergy and Infectious Diseases (NIAID)	Clinical trial phase III (NCT04470427)
2.	BNT162	LNP-encapsulated mRNA vaccine targeting whole S-protein and RBD domains	2	Intramuscular	BioNTech SE, Germany, Pfizer Inc., New York, and Fosun Pharmaceutical, China	Clinical trial phase III (NCT04368728)
3.	nCoVsaRNA	LNP-encapsulated saRNA	2	Intramuscular	Imperial College of London and TriLink BioTechnologies, San Diego	Clinical trial phase I (ISRCTN17072692)
4.	CvnCoV	LNP-encapsulated mRNA	2	Intramuscular	CureVac AG, Germany	Clinical trial phase II (NCT04515147)
5.	ARCT-021	mRNA	NA	Intramuscular	Arcturus Therapeutics Holdings Inc., San Diego and Duke-NUS Medical School (Duke-NUS), Singapore	Clinical trial phase I/II (NCT04480957)
6.	ARCoV	mRNA	2	Intramuscular	People's Liberation Army (PLA) Academy of Military Sciences and Walvax Biotech Co. Ltd., China	Clinical trial phase I (ChiCTR2000034112)
7.	GX-19	DNA	2	Intramuscular	Genexine, Inc., South Korea	Clinical trial phase I/II (NCT04445389)
8.	ZyCoV-D	DNA	3	Intradermal	Cadila Healthcare Ltd., Ahmadabad, India	Clinical trial phase I/II (CTRI/2020/07/026352)
9.	INO4800	DNA plasmid vaccine with electroporation	2	Intradermal	Inovio Pharmaceuticals, Pennsylvania	Clinical trial phase I/II (NCT04447781; NCT04336410)
10.	AG0301	DNA vaccine with adjuvant	2	Intramuscular	Takara Bio Inc., Japan, Osaka University and AnGes, Inc. group	Phase I/II clinical trial (NCT04463472)
11.	NA	Non-replicating adenovirus type5 (Ad5) vector	1	Intramuscular	CanSino Biologics Inc., China and Beijing Institute of Biotechnology	Clinical trial- II (ChiCTR200003178)
12.	AZD1222 (ChAdOx1)	Non-replicating chimpanzee based Ad5 vector vaccine targeting S-protein	1	Intramuscular	University of Oxford, Oxford and AstraZeneca Plc, Cambridge	Paused
13.	Gam-COVID-Vac Lyo and Gam-COVID-Vac	Non-replicating Ad5 vector	1	Intramuscular	Gamaleya Research Institute, Russia	Registered for clinical use.
14.	Ad26COVS1	Non-replicating Ad5 vector	2	Intramuscular	Janssen Pharmaceutical Companies	Clinical trial phase I/IIa (NCT04436276)
15.	Ad5-nCoV	Non-replicating Ad5 vector	2	Intramuscular/mucosal	Institute of Biotechnology, Academy of Military Medical Sciences, PLA of China	Clinical trial phase I (NCT04552366)
16.	VXA-CoV2-1	Non-replicating Ad5 adjuvanted Oral Vaccine platform	2	Oral	Vaxart Inc., USA	Clinical trial phase I (NCT04563702)
17.	GRAd-CoV2		1	Intramuscular	ReiThera Srl, Italy	Clinical trial phase I (2020-002835-31)

TABLE 1 (Continued)

SN	Vaccine name	Vaccine platform	No. of doses	Mode of administration	Manufacturer/collaborator	Current status
		Replication-defective gorilla adenoviral (GRAd) vector				
18.	NA	Non-replicating MVA-SARS-2-S vector vaccine	2	Intramuscular	Ludwig-Maximilians - University of Munich, Germany	Clinical trial phase I (NCT04569383)
19.	TMV-083	Measles-vector based replicating vaccine	1 or 2	Intramuscular	Institut Pasteur, Paris, Themis, Australia and University of Pittsburgh, Pennsylvania	Clinical trial phase I (NCT04497298)
20.	DeINS1-2019-nCoV-RBD-OPT1	Flu-vector based replicating vaccine	1	Intranasal	Beijing Wantai Biological Pharmacy Enterprise Co. Ltd., China and Xiamen University, China	Clinical trial phase I (ChiCTR2000037782)
21.	NA	Inactivated virus	2	Intramuscular	China National Pharmaceutical Group, Sinopharm and Wuhan Institute of Biological Products	Clinical trial phase IV (NCT04053010)
22.	NA	Inactivated virus	2	Intramuscular	China National Pharmaceutical Group, Sinopharm and CNBG-Beijing Institute of Biological Products Co. Ltd., Beijing	Clinical trial phase IV (NCT04053010)
23.	NA	Inactivated virus	2	Intramuscular	Institute of Medical Biology, Chinese Academy of Medical Sciences	Clinical trial phase I/II (NCT04470609)
24.	PiCoVacc	Inactivated virus vaccine targeting N- and RBD- domain proteins	2	Intramuscular	Sinovac Research and Development Co. Ltd., China	Clinical trial phase III (NCT04456595)
25.	BBV152 (COVAXIN)	Inactivated virus	2	Intramuscular	Bharat Biotech, Hyderabad, India	Clinical trial phase I/II (NCT04471519)
26.	QAZCOVID-IN	Inactivated virus	2	Intramuscular	Research Institute for Biological Safety Problems, Rep of Kazakhstan	Clinical trial phase I/II (NCT04530357)
27.	NVX-CoV2373	SARS-CoV-2 S-protein nanoparticle along with Matrix M adjuvant vaccine targeting S-protein	2	Intramuscular	Novavax Inc., USA	Clinical trial phase III (2020-004123-16)
28.	COVAX	Advax-CpG55.2 adjuvant platform	1	Intramuscular	Vaxine Pty Ltd., Adelaide and Medytox Inc., Korea	Clinical trial phase I (NCT04453852)
29.	SCB 19	S-Trimer	2	Intramuscular	Clover Biopharmaceuticals and Dynavax, USA	Clinical trial phase I (NCT04405908)
30.	MVC-COV1901	S-2P protein and CpG 1018 based vaccine	2	Intramuscular	Medigen Vaccine Biologics Corp., Taiwan and Dynavax Technologies Corp., USA	Clinical trial phase I (NCT04487210)
31.	NA	Subunit vaccine targeting RBD domain proteins	2 or 3	Intramuscular	Anhui Zhifei Longcom Biopharmaceutical Inc. and Institute of Microbiology, Chinese Academy of Sciences	Clinical trial phase III (NCT04466085)
32.	NA	S-protein MF59 adjuvant-based vaccine	2	Intramuscular	University of Queensland, Australia and CSL Ltd., Melbourne	Clinical trial phase I (ACTRN12620000674932p)

(Continues)

TABLE 1 (Continued)

SN	Vaccine name	Vaccine platform	No. of doses	Mode of administration	Manufacturer/collaborator	Current status
33.	NA	Subunit vaccine targeting RBD domain proteins	2	Intramuscular	Kentucky Bioprocessing Inc., USA	Clinical trial phase I/II (NCT04473690)
34.	EpiVacCorona	Peptide subunit	2	Intramuscular	FBRI SRC VB VECTOR, Rosptrebmadzor, Koltsovo	Clinical trial phase I (NCT04525757)
35.	NA	RBD (baculovirus production expressed in Sf9 cells)	2	Intramuscular	West China Hospital, Sichuan University	Clinical trial phase I (ChiCTR2000037518)
36.	FINLAY-FR-1	RBD + Adjuvant based vaccine	2	Intramuscular	Instituto Finlay de Vacunas, Cuba	Clinical trial phase I/II (IFV/COR/04)
37.	NA	S-protein (RBD (baculovirus production))	2	Intramuscular	Sanofi S.A., France, and GlaxoSmithKline Plc. England	Clinical trial phase I/II (NCT04537208)
38.	UB-162	S1-RBD-protein	2	Intramuscular	COVAXX, United Biomedical Inc., Asia	Clinical trial phase I (NCT04545749)
39.	CoVacc-1	SARS-CoV-2 HLA-DR peptide; Protein subunit vaccine	1	Subcutis	University Hospital Tuebingen	Clinical trial phase I (NCT04546841)

complications for unknown reason. The Sputnik V (Gam-COVID-Vac Lyo and Gam-COVID-Vac) are registered on August 12, 2020, for clinical use, this vaccine was in late III clinical trial at the time of registration and no data published to date, therefore efficacy and safety of this therapy was questioned by the scientists all over the globe. In summary, the COVID-19 vaccine development and trials to materialize quickly and provide an effective vaccine against COVID-19 is one of the major concerns in every country under the global pandemic situation.

Vaccinations are one of the most successful forms of prophylactic approach for disease prevention. Since the burden of the infectious viral epidemic situation of COVID-19 remains high, especially in developing countries; therefore, future strategies need to meet the challenges of extending the use of the existing vaccines, invent new technology for vaccine delivery, and developing new vaccines. Future vaccines also need to present more or multiple targets for better and long-lasting immunization even in a single dose. Moreover, working on edible vaccine materials, needle-free skin patches and microneedle injection technologies to get the vaccine through the skin without discomfort will be an additional challenge associated with vaccine formulation. Recently, the PittCoVacc vaccine against COVID-19 infection is developed by the University of Pittsburgh which utilizes a microneedle vaccine platform for skin dissolvable patches and insertion of S-protein. This type of vaccine platform will assist in multidose administration without any discomfort. The frequent genetic evolutions of the pathogen will also open doors for personalized vaccines requiring well equipped manufacturing unit at low cost and is easily assessable. The peptide-based immunotherapy has also enhanced future study in multi-epitope peptide-based vaccines development against SARS-CoV-2 exhibiting higher immunogenicity with appropriate adjuvants compare to conventional peptide vaccines. The immunoinformatic approaches of epitope vaccines synthesis promoted the generation of laboratory free, biologically safe, and highly selective multiple vaccine candidates against SARS-CoV-2,<sup>89,90</sup> yet future studies are recommended to justify the efficacy and therapeutic intervention of predicted epitopes for COVID-19 treatment.

There are no effective treatment therapies that are available for SARS-CoV-2 infection and social distancing and lockdown are economically irrational, unfeasible, and costly. Therefore, it is indispensable to implement immune-enhancing strategies and developing effective vaccines against COVID-19. The priority for vaccine development on the basis of previously reported vaccine platforms would further facilitate rapid progression towards vaccine formulation; however, bringing successful vaccines against SARS-CoV-2 for public health is still a long way to go.

## 6 | CONCLUDING REMARKS

This review article provides an outline of global research and development on COVID-19-related preventive vaccines based on WHO database collection and focused on current pharmaceutical

market status in concern of SARS-CoV-2. An overview of probable immunogenic pathways is discussed with a particular focus on vaccine strategies targeting complex molecular interactions induced by immune cells against SARS-CoV-2 coronavirus. The vaccine-repurposing efforts are summarized in the review suggesting the development of potential vaccine platforms that are previously prescribed and found effective in SARS-CoV, MERS, Ebola, influenza, and hepatitis C virus infections. The information given in this report is an excellent intellectual summary to promote future research in prophylaxis and prevention through new vaccine discovery for COVID-19. Due to less published data on the development of vaccines against COVID-19, this study needs major attention toward significant efforts to be made in advancing efficient and accurate vaccine development.

Over the period of the first 6 months in 2020, a concerted effort to develop reliable vaccines against the COVID-19 outbreak has been made and it is very necessary in terms of reducing overwhelming impacts on the worldwide healthcare system. Therefore, on the basis of SARS-CoV and MERS vaccines, COVID-19 vaccine development was commenced immediately disease outbreak. Currently, more than 100 vaccine candidates are under trials where viral nucleic acids, inactivated viruses, and protein subunits vectors-based vaccines are offering effective and preventive measurements against COVID-19. Multiple vaccination strategies are proposed to date, targeting S-protein as an ideal candidate to stimulate immune response and elicit cross-protective immunity against SARS-CoV-2 coronavirus. Though, development of inactivated or live attenuated vaccines take longer time and further to evaluate their efficacy and safety in human is a time-consuming process. But the scenario of COVID-19 pandemic, collaborative efforts of academia and industry ambitiously accelerated the timeline of vaccine development and thereby generating safety data in clinical trials.

However, each of these multistage vaccine developments requires precautionary measures incorporating additional manufacturing steps with toxicological studies. Furthermore, the efficacy of data collection before handing over to regulatory agencies to commence clinical development is important. The upcoming months are a critical period to determine whether a warmer environment in the Northern hemispheres will reduce COVID-19 transmission or its independent nature of virus incubation. The former situations have already called for rapid vaccine development, however, the challenges remain the same. The major hurdle for COVID-19 vaccine development can be the frequent mutation rate in RNA virus, as of now asymptomatic COVID-19 patients also reported positive tests. If the mutation rate continues in coronavirus evolution and whether genome variability has occurred, then the virus would be resistant to these developed vaccines. The previous study on 86 genomes of SARS-CoV-2 cases revealed eight missense mutation in S-protein, three mutations in RBD domain,<sup>91</sup> and 382-nt deletion in ORF8 of SARS-CoV-2.<sup>92</sup> These studies supported rapid evolution of the SARS-CoV-2 coronavirus; therefore, it is advisable to move to personalized vaccines on the basis of

geography and genetic homogeneity. The key issue of vaccine development is time consuming clinical trials and recruiting volunteers for mega-scale trials are always a hurdle for the authorities. The difficulty in shortening the time of vaccine discovery, production, and clinical development while maintaining the efficacy of the vaccine for positive results are still the major challenges. The reverse and synthetic vaccinology also require sophisticated equipment which is very expensive and will increase the price for the formulated vaccines. The development of personalized vaccines will also be very time-consuming since the genetic analysis of each person in a pandemic situation is very difficult. A new coronavirus epidemic in every decade in 21st century has already occurred including SARS in the 2000s, MERS in the 2010s, and recently COVID-19. This frequent outbreak suggests manufacturing and storing advanced coronavirus vaccines at a mass level and targeting new alternates in vaccine development with global priority. As a matter of fact, the current situation of the COVID-19 pandemic is serving as a beacon to react faster and prepare for the next wave of the virus across humans.

#### ACKNOWLEDGMENTS

The work is financially supported through Extramural Research grant (file no. EMR/2016/007564) by Science and Engineering Research Board, Government of India and Technology Development Program (TDP) (TDP/BDTD/33/2019), DST, Government of India and Biotechnology Industry Research Assistance Council (BIRAC) (File No. BT/IIPE0211/02/16) which are highly acknowledged.

#### CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

#### AUTHOR CONTRIBUTIONS

Utkarsh Jain, Nidhi Chauhan and Shringika Soni wrote the original draft, performed formal analysis, conceptualization, and literature investigation. Abhinandan Gupta performed the literature investigation and designed the images. Mohammad Aslam provided clinical insight of the COVID-19 vaccine and performed formal analysis. Nidhi Chauhan and Utkarsh Jain conceptualized and reviewed, edited the manuscript, supervised, and acquired the funding.

#### DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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**How to cite this article:** Chauhan N, Soni S, Gupta A, Aslam M, Jain U. Interpretative immune targets and contemporary position for vaccine development against SARS-CoV-2: A systematic review. *J Med Virol*. 2021;93:1967-1982.  
<https://doi.org/10.1002/jmv.26709>