

## REVIEW

# COVID-19: A systematic review and update on prevention, diagnosis, and treatment

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## Abstract

Since the rapid onset of the COVID-19 or SARS-CoV-2 pandemic in the world in 2019, extensive studies have been conducted to unveil the behavior and emission pattern of the virus in order to determine the best ways to diagnosis of virus and thereof formulate effective drugs or vaccines to combat the disease.

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The emergence of novel diagnostic and therapeutic techniques considering the multiplicity of reports from one side and contradictions in assessments from the other side necessitates instantaneous updates on the progress of clinical investigations. There is also growing public anxiety from time to time mutation of COVID-19, as reflected in considerable mortality and transmission, respectively, from delta and Omicron variants. We comprehensively review and summarize different aspects of prevention, diagnosis, and treatment of COVID-19. First, biological characteristics of COVID-19 were explained from diagnosis standpoint. Thereafter, the preclinical animal models of COVID-19 were discussed to frame the symptoms and clinical effects of COVID-19 from patient to patient with treatment strategies and in-silico/computational biology. Finally, the opportunities and challenges of nanoscience/nanotechnology in identification, diagnosis, and treatment of COVID-19 were discussed. This review covers almost all SARS-CoV-2-related topics extensively to deepen the understanding of the latest achievements (last updated on January 11, 2022).

#### KEYWORDS

biomaterials, coronavirus, COVID-19, nanotechnology, pandemic, SARS-CoV-2

## 1 | INTRODUCTION AND BACKGROUND

Based on the World Health Organization (WHO) declaration, the total reported cases of the ongoing global pandemic, coronavirus disease 2019 (COVID-19), naming Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) via the International Committee on Taxonomy of Viruses (ICTV), as of 5:48 pm CEST, January 11, 2022, have been more than 311,316,780 confirmed cases with 5,514,603 deaths, with a very sharp increase in the number of new cases because of accelerated Omicron variant transmission (designated by WHO on November 26, 2021) in the world.<sup>1</sup> This new emerging zoonotic reservoir virus with one or more probable mammal intermediate hosts has received the human transmission ability like the other six Coronaviruses family members, especially alpha and beta subfamilies (HCoV-OC43, HCoV-NL63, HCoV-HKU1, HCoV-229E, MERS-CoV, SARS-CoV, and 2019-nCoV [SARS-CoV-2]).<sup>2</sup> In the 20th century, the three of seven members of Coronaviruses family (SARS-2003 [case-fatality rate: 10–12%], MERS-2012 [case-fatality rate: 36%], and COVID-19–2020 [case-fatality rate: estimated lower than other]) have been the cause of the highest death rate from pneumonia after lower respiratory tract replication. Although COVID-19 has lower case fatality, its transmissibility was proved to be higher than other respiratory viruses based on R0 value calculation. Age, sex, having risk factors, and country regions can be

affected by the case-fatality rate.<sup>3</sup> New reports declare the effect of virus genome mutation on the pathogenicity and immunogenicity of viruses.<sup>4,5</sup> Transmission of the virus can occur through human respiratory droplets or contact with virus-infected surfaces.<sup>6</sup> Transmission through blood transfusion, solid organ transplantation, and mother-to-neonate vertical transmission have no convincing evidence and need further studies. The possibility of transmitting the virus through environmental factors such as virus-contaminated wastewater and airborne dust is under investigation. In this situation, the management of transmission by prevention and screening of COVID-19 is the best advice. The development of high-powered and automated techniques for virus monitoring is required.<sup>7–11</sup> Dry cough, sore throat, fatigue, runny nose, and severe headache are the COVID-19 ordinary symptoms. Possible complications are fever, severe breath shortness, and digestive symptoms, such as diarrhea, severe pneumonia, hemoptysis, and anosmia. Subsequently, multiorgan damage can happen.<sup>12</sup> Symptoms and case history reviews are the first level of medical detection of the virus. Reverse transcription polymerase chain reaction (RT-PCR) for disease in its early stages and chest computed tomography (CT) scans for severe stages are confirmable detection ways.<sup>3</sup>

The serological test has not been confirmed yet for the detection of COVID-19 by medical policymakers. Despite the sensitivity of immunoglobulin M (IgM) and immunoglobulin G (IgG) antibody detection tests being



77% and 83%, and the specificity of them being 100% and 95%, immunoglobulin secretion has not had the same pattern in all cases. To enhance the speed and efficiency of detection, researchers are developing point of care (POC) tests. Potential strategies to combat COVID-19 include interferon (IFN) therapies, monoclonal antibodies (mAbs), vaccine production, peptides, oligonucleotide-based therapies, small molecules or natural remedies of conventional medicine, and plasma therapy. The time to develop a *de novo* small molecule drug is more than 6 years, and in the best time, it takes at least 2 years. Vaccines are produced faster, about 1–2 years. Immune system supportive antibodies can be used to treat viral diseases, although the period of their production and development is usually lasting several years.<sup>13</sup> The promising strategy in the current situation is the new use of old drugs, and its purpose is to discover new fields for previously authorized drugs.<sup>14</sup> The main benefit of reusing the drug is fast development due to the vast knowledge about this drug's behavior in humans. The basis of the disease is on immunology and genetics, so the role of controversial factors, such as IL6, while affecting severe respiratory symptoms, should not be overlooked in controlling inflammation, virus removal, T cell maturation, and acquired immunity. Therefore, the use of any permitted immunosuppressive agents is not allowed and must be checked.<sup>15,16</sup> Increasing flu vaccine absorption or strengthening public health interventions may facilitate the management of respiratory outbreaks during the flu season and compensate for the lack of diagnostic resources. However, how to increase the coverage of influenza vaccination remains a challenge. Public health decision-makers and policymakers need to adopt informed strategies to improve flu vaccination. Difficult months lie ahead for researchers, medical staff, and government rulers.<sup>17</sup>

## 2 | BIOLOGICAL PROPERTIES

In this section, the biological properties of COVID-19 referred to in Figure 1 are examined.

### 2.1 | Genomics and proteomics

SARS-CoV-2 is a 100 nm enveloped virus with about one femtogram mass containing a positive-sense, linear single-strand RNA with a length of about 29,800 base pairs. Two-thirds of the genome typically codes for nonstructural proteins, and a third of the genome encodes structural proteins.<sup>18</sup> Four main structural proteins, which construct the virus, are membrane (M), envelope (E), nucleocapsid (N), and spike (S) proteins. The spike protein is

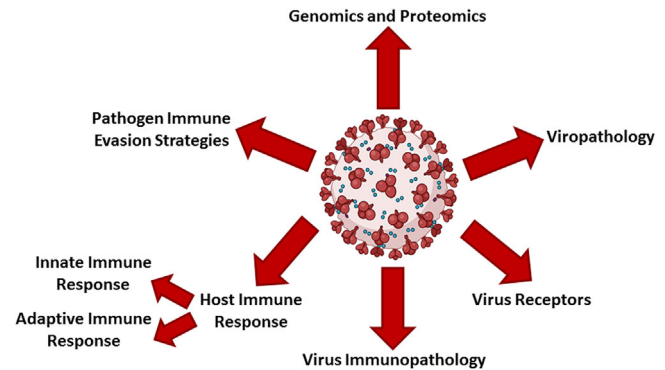


FIGURE 1 An overview of the biological properties of COVID-19. The figure is created with BioRender.com

the most noteworthy antigen on the virus's surface and has a trimmer structure with two subunits called S1 and S2. S1 possesses more important role with more diversity between species. The S2 subunit plays the virus membrane fusion role and is more protected. The receptor-binding domain (RBD) sequence of the spike S1 subunit, which the virus binds to the receptor through this domain, is the primary and specific target of the virus in producing diagnostic kits and drugs.<sup>19</sup> There are more than 5000 reports examining the complete genomes of the virus and their mutations. These reports show that 1100 nucleotides have undergone point mutations.<sup>20</sup> It is reported that there are variants in some virus gene regions, which has changed the level of amino acid residues to about 12 nucleotides. The mutation rate of this virus is low and is about  $10^{-6}$ /site/cycle, while the mutation of similar viruses, such as influenza, is  $3 \times 10^{-6}$ /site/cycle.<sup>21</sup> The presence of endonuclease and exonuclease as proofreading enzymes and RNA-dependent RNA polymerase enzyme (RdRp) are the main reasons for these mutations.<sup>22</sup> Bat coronavirus (RATG13) and Penguin coronavirus, respectively, with 96% and 91% similarity, have the closest genome structure with SARS-CoV-2.<sup>23,24</sup> There are emerging new variants, which are reported from the beginning of the pandemic till now, which are driven of the heritable mutations of SARS-CoV-2.<sup>25</sup> In Table 1, these variants are listed in order of variant of concern (VOC), variant under monitoring (VUM), and variant of interest (VOI). Jackson and colleagues show evidence of recombination in SARS-CoV-2. In their study, four from eight identified recombinant-origin viruses had onward transmission evidence.<sup>26</sup>

### 2.2 | Viropathology

During cell fusion, the virus enters the cell via superficial phospholipids. The incubation period of the virus lasts about 10 h, followed by an assembly process of about

TABLE 1 Heritable mutations of SARS-CoV-2

| Variants                       | Name/first detected      | Lineage                   | Country of first detection | Mutations on S protein  | Signature mutations                      | References |
|--------------------------------|--------------------------|---------------------------|----------------------------|---|--|------------|
| Variant of concern (VOC)       | Alpha<br>December 2020   | B.1.1.7                   | UK                         | Δ69/70, Δ144/145, H69, V70, Y144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H  | N501Y                                    | 27,28      |
|                                | Beta<br>December 2020    | B.1.351                   | South Africa               | D614G, L18F, D80A, D215G, Δ242-244, R246I, K417N, E484K, N501Y, and A701V   | Δ242-244, R246I, K417N, E484K, and N501Y | 27–29      |
|                                | Gamma<br>November 2020   | P.1 (B.1.1.28.1)          | Japan/Brazil               | D614G, L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y, and T1027I   | K417T, E484K, and N501Y                  | 28,30,31   |
|                                | Delta<br>May 2021        | B.1.617.2                 | India                      | T19R, E156, F157, R158G, L452R, T478K, D614G, P681R, and D950N  | L452R and T478K                          | 32–34      |
|                                | Omicron<br>November 2021 | B.1.1.529                 | South Africa               | Δ69/70, A67V, H69, V70, T95I, S:G142, V143, Y144, Y145D, N21I, L212I, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, and N969K | H655Y, N679K, P681H, E484A, and N501Y    | 35,36      |
| Variant under monitoring (VUM) | Epsilon<br>January 2021  | B.1.427/B.1.429 (CAL.20C) | California                 | S13I, W152C, L452R, and D614G   | L452R, S13I, and W152C                   | 37–39      |
|                                | Eta<br>December 2020     | B.1.525                   | South Africa               | Q52R, A67V, ΔH69/V70, ΔY144/145, E484K, D614G, Q677H, and F888L   | Δ69/70, Δ145, E484K, and Q677H           | 40         |
|                                | Lota<br>November 2020    | B.1.526                   | USA                        | L5F, T95I, D253G, D614G, A701N, and S477N or E484K  | L5F, T95I, D253G, S477N, or E484K        | 41         |
|                                | Kappa<br>October 2020    | B.1.617.1                 | India                      | D614G, G142D, E154K, L452R, E484Q, P681R, Q1071H, and H1101D  | L452R and E484Q                          | 36,40,42   |
|                                | C.1.2<br>May 2021        | B.1.1.1.1.2               | South Africa               | E484K and E484Q<br>L452R s  | E484Q                                    | 27         |
| Variant of interest (VOI)      | Lambda<br>August 2020    | B.1.1.1.37<br>C.37        | Peru                       | Δ247-253, G75V, T76I, R246, S247, Y248, L249, T250, P251, G252, D253N, L452Q, F490S, D614G, and T859N   | L452Q and T859N                          | 43–45      |

(Continues)

TABLE 1 (Continued)

| Variants | Name/first detected    | Lineage          | Country of first detection | Mutations on S protein  | Signature mutations     | References |
|----------|------------------------|------------------|----------------------------|---|-------------------------|------------|
|          | Theta<br>February 2021 | P.3 (B.1.1.28.3) | Philippines                | D614G, ΔLGV141-143, E484K, N501Y, P681H, E1092K, H1101Y, and V1176F | E484K, N501Y, and P681H | 46,47      |
|          | Mu<br>January 2021     | B.1.621          | Kolumbien                  | T95I, Y144S, Y145N, R346K, E484K, N501Y, D614G, P681H, and D950N    | Y144S and Y145N         | 27         |
|          | Zeta<br>July 2021      | P.2 (B.1.1.28.2) | Brazil                     | D138Y, R190S, E484K, H655Y, T1027I, and V1176F                      | E484K and V1176F        | 46,47      |

10–12 h, in which finally the  $10^3$ /cell virus lyses the cell that leads to infection. The binding between SARS-CoV-2 and human cellular receptors is much stronger than the binding of their same family member viruses to these receptors. Insertion of 12 nucleotides among the sequence of S1 and S2 results in the formation of a protease cut site. This cut site is individual for the SARS-CoV-2 virus and is effected on higher pathogenicity of it.

### 2.3 | Virus receptors

The angiotensin-converting enzyme 2 (ACE2) cell receptor is an intramembrane protein with the hemostatic role in balancing the impacts of ACE on the cardiovascular system. In such virus infection, virus attaching to ACE2 leads to its endocytosis and decreases the amount of surface ACE2 on the endothelial cells, which can upset the balance between ACE and ACE2 and subsequently increase angiotensin II levels. Angiotensin II, commonly known as a vasoconstrictor, promotes inflammation by activating enzymes, such as a disintegrin and metalloprotease 17 (ADAM17).<sup>48,49</sup> In addition to ACE2, other receptors for SARS-CoV-2 entry were described, such as another member of the coronaviruses family-like NL-63. These receptors consist of CD209L (called L-SIGN and CLEC4M) and CD209 (called DC-SIGN), both of them are cell adhesion receptor members, such as the immunoglobulin family.<sup>50</sup> The CD209L is overexpressing in type II alveolar cells and lung endothelial cells and the CD209 is expressing in dendritic cells (DCs) and tissue-dwelling macrophages.<sup>51</sup> Another receptor of this virus is Basigin, also called CD147 or EMMPRIN (extracellular matrix metalloproteinase inducer). This membrane glycoprotein (belonging to the immunoglobulin family) serves as a ligand for virus spikes. In the discussion of blocking receptors, the specificity of inhibitory drugs (antireceptors) has been based on the spatial epitopes of these surface glycoproteins.<sup>52,53</sup>

### 2.4 | Virus immunopathology

The resemblance of the SARS-CoV-2 virus with its family-related viruses suggested that the immunopathogenic response due to host–pathogen interaction may be similar. Lack of adequate immune response in combat with SARS-CoV-2 can affect the disease severity. In less than 20% of patients leading to severe symptoms like acute respiratory distress syndrome, loss of lung organs, respiration, shock, and less than 2.4% of them lead to death.<sup>54</sup> These conditions can be due to the immune system's malfunction in blocking and regulating the immune response. Overproducing of the proinflammatory cytokines (such as interleukin-1 [IL-1], IL-2, IL-6, IL-8, IL-17, and tumor necrosis factor-alpha [TNF-alpha]) and chemokines (CXCL10 and CCL2) by immune cells leads to cytokine storms. The second mechanism observed in these patients, similar to the MERS virus, is lymphopenia, especially T cells reduction, which can occur under different approaches. The lode of the virus, the age, health of the immune system, and the presence of chronic diseases due to imbalance in proinflammatory and anti-inflammatory cytokines, are very important influential factors. Induction the expression of these cytokines and stimulation of their signal cascade is performed directly and indirectly by viral proteins (especially S and N proteins). Adequate knowledge about the disease's immunopathogenesis can be affected in selecting potential targets to increase the immune response.<sup>55,56</sup>

### 2.5 | Host immune response

#### 2.5.1 | Innate immune response

This type of immune response has a vital responsibility in protecting or failing the responses against the virus. Single-stranded RNA (ssRNA) or double-stranded RNA of these kinds of viruses as pathogen-associated molecular

patterns engage in innate immune cells like neutrophils and monocytes—macrophages via the cytosolic RNA sensor (RIG-I/MDA5) as intracellular or endosomal RNA receptors (TLR3 and TLR7) pattern recognitions. This engagement stimulates proinflammatory cytokines expression by launching downstream signaling cassettes, such as NF- $\kappa$ B (nuclear factor kappa light chain enhancer of activated B cells) and IRF3 (IFN regulatory factor 3). Neutrophils, C-reactive protein, and serum IL-6 increasing and also total lymphocytes decreasing are other cases of infection with this virus.<sup>57</sup> Increasing the proinflammatory cytokines can stimulate the neuroendocrine system, secreting glucocorticoids, and other peptides, subsequently impairing the immune response.<sup>58</sup>

### 2.5.2 | Adaptive immune response

Both humoral and cellular immunity will act against the virus. Humoral immunity works by producing neutralizing antibodies to limit reinfection by targeting viral antigens. The most potential antibodies after the isotype switching are IgG subtypes. Excessive secretion of these antibodies is present in acute conditions that can have adverse effects. One of these complications is an antibody-dependent enhancement, happening in some types of viral infections. In this mechanism, the antibody itself becomes a way for the virus to attach to the cell and accelerate its entry and increase inflammation.<sup>59</sup> Cellular immunity through cytotoxic T cells kills virus-infected cells, and T helper type 1 (Th1) cells play a critical role in immunization against the virus. S, M, N, and E proteins are vital molecules in the development of immunogenicity, but also, S protein is involved in both humoral and cellular responses.<sup>56,60</sup>

### 2.6 | Pathogen immune evasion strategies

Most viral proteins, especially M, N, and S, can block the signaling pathway of type I IFN production and suppress the host's innate antiviral response. On the other hand, reducing antigen presenting by lowering the expression of major histocompatibility complex class I and class II (MHC-I and II) is also another mechanism of the virus escaping from the adaptive immune response. Immune exhaustion, viral mutations, and immune deviation can be the other potential immune evasion methods.<sup>57,60,61</sup>

## 3 | DETECTION

Section 3 reviews the different COVID-19 detection methods used so far. These methods can be seen as categorized in Figure 2.

### 3.1 | Molecular detections of COVID-19

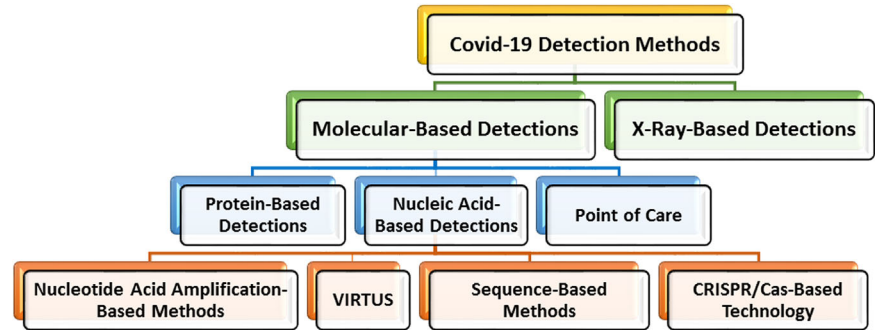
COVID-19 diagnostic instruments are usually available based on molecular detection in the market. Molecular detection, which is classified into two main groups based on nucleic acid and protein base methods, is the most powerful technology for detecting SARS-CoV-2 so far.<sup>62–64</sup> Some of which are laboratory tests, such as next-generation sequencing (NGS), PCR, enzyme-linked immunosorbent assay (ELISA), chemiluminescent immunoassay (CLIA),<sup>65</sup> and some others are POC tools<sup>66</sup> like GICA.<sup>67</sup> Besides nucleic acid and protein detection, numerous techniques are established to detect the whole virus in specimens, such as flow-virometry.<sup>68</sup>

#### 3.1.1 | Nucleic acid-based detections

##### *Nucleotide acid amplification-based methods*

Detection of the SARS-CoV-2 viral genome, consisting of ssRNA, is effectively done by quantitative reverse transcription PCR (RT-qPCR), which is commonly used as the molecular diagnostic gold standard.<sup>69,70</sup> The tests contain nucleic acid extraction/purification from samples and finally RT-PCR.<sup>69</sup> The approved detection kits are based on different viral targets, including RdRp, envelope (E), spike (S), open reading frame (ORF1ab) 1a, and nucleocapsid protein (N).<sup>70,71</sup> The LoD gets to levels less than 10 genome copies per reaction (0.5 cp/ $\mu$ l), depending on the chosen kits and PCR instrument. Although RT-qPCR is sensitive and reliable, it takes at least 2 h. It needs a specific tool, trained technicians, and well-equipped laboratories, limiting its application in underequipped laboratories or on-site.<sup>72,73</sup> In addition, in order to increase the sensitivity and accuracy, several practical considerations like sample quality, viral load, sampling methods, and sample source must be taken into account when performing diagnostic assays by RT-qPCR.<sup>74</sup> A method called RTLAMP (reverse transcription loop-mediated isothermal amplification (IA)) has been developed for quick and easy detection of SARS-CoV-2 nucleic acid.<sup>75</sup> The LAMP reaction, which is a reliable and rapid assay with the low equipment cost, is performed at constant temperature (usually 65°C) for 20 min. The testing outcome is detected by a color change, which can be analyzed directly by the naked eye. Due to the fact that this method uses four sets of primers that target ORF1ab, S, and N genes of SARS-CoV-2, its specificity is high. The detection limit of the technique was 80 copies of viral RNA per milliliter sample.<sup>76</sup> Another technique based on IA is recombinase polymerase amplification (RPA), which does not require a PCR machine. Amplification is performed on a specific combination of enzymes and proteins followed by fluorescence measurements by tube scanners to detect.

**FIGURE 2** An overview of the COVID-19 detection methods



The whole process is performed at a constant temperature (between 39 and 42°C) under 20 min and the clinical sensitivity and specificity of this about SARS-CoV-2 are 98% and 100%, respectively.<sup>77</sup> These robust, accurate, and simple operation methods have solved RT-qPCR problems, such as time-consuming and expensive equipment. Nonetheless, there is a need for the one-step process to accomplish RNA amplification directly from a throat swab sample without RNA extraction, which can be used as a rapid on-site test.<sup>78</sup>

#### *CRISPR/Cas-based technology*

The clustered regularly interspaced short palindromic repeats (CRISPR)-based nucleic acid recognition systems incorporate RPA with CRISPR–Cas12/13 enzymology for particular target detection.<sup>79,80</sup> In one study, SHERLOCK (high-sensitivity enzymatic reporter unlocking) technology was developed, which targets S and ORF1ab genes. In this method, first, amplification is performed using RPA, and then targeting of preamplified viral RNA sequence using Cas13, leading to generate fluorescence and detection of COVID-19. To enable SHERLOCK for rapid detection assay, they introduced STOPCovid (SHERLOCK Testing in One Pot), performed at a single temperature without the need for sample extraction and a simple visual using lateral flow readout. LOD of this method is 100 copies of the viral genome per reaction.<sup>81</sup> Currently, two of the CRISPR-based SARS-COV-2 diagnostic tests have received FDA emergency use authorization that are now available for field use, and will become more common.<sup>82</sup> Today, despite new and valuable approaches to SARS-CoV-2 detection, the vast majority of tests worldwide are still performed using PCR-based methods. To date, among available molecular-based assays for the detection of SARS-CoV-2 in the United States, 90% are PCR-based methods, 6% are IA assays, 2% are hybridization techniques, and 2% are CRISPR technology.<sup>83</sup>

#### *Sequence-based methods*

Whole-genome sequencing is another technology to identify the Deoxyribonucleic acids (DNAs), which can be uti-

lized for detection and evaluation of mutational properties of the SARS-CoV-2.<sup>84</sup> Because of being costly, time-consuming, and intricate, this method is unsuitable for crucial and large-scale testing. So, new strategies based on sequencing were developed. A laboratory in Wuhan developed a novel approach, nanopore target sequencing. At the primary step, the amplification of different SARS-CoV-2 gene fragments is performed. The nanopore platform is involved in the sequencing of the amplicons. Nanopore system can sequence and evaluate the outputs at the same time, letting us to detect SARS-CoV-2 in short time. Also, by adding multiple characteristic primers, it can be used for simultaneously analyzing and identification of various types of viruses, including influenza.<sup>78</sup> In one of the published researches, INSIGHT (Isothermal NASBA sequencing-based high-throughput test) was proposed. This is a two-phase approach and is performed by an isothermal nucleic acid sequence-based amplification (NASBA) reaction followed by NGS. The sensitivity of this method is high (10–100 copies per reaction), and confirming the rapid results of the first stage by NGS analysis improves the test accuracy in a high-throughput way.<sup>85</sup> Another strategy is massively parallel diagnostic assays, which combines reverse transcriptase (RT), PCR, and NGS for detection. RT primers with highly unique barcode sequences link to three amplicons (N1 and N2 SARS-CoV-2, RNase P human control). After the RT reaction, the cDNA of patient samples is pooled together and amplified in a single PCR, followed by NGS. Using patient-specific primers at the RT step enables this method simultaneously to carry out thousands of viral RNA measurements in a single assay.<sup>86</sup> These methods are transportable, scalable, accurate, rapid, and cost-effective strategies for detecting SARS-CoV-2; however, further development is needed to bring them into practical use.

#### *VIRTUS*

A sensor named VIRTUS (Viral Transcript Usage Sensor) was developed for detecting and quantifying mRNA transcripts in the data of traditional human RNA-seq data. VIRTUS is applied to identify the cells sheltering activated

viruses and not the copy number of the virus, the composition of multiple viruses in a cell, and the expression differences between infected and uninfected cells.<sup>87</sup>

### 3.1.2 | Protein-based detections

Immunological assays have also been used as COVID-19 detection. They have been categorized in antigen base and antibody-based (serological) detection. Antigen testing has been used as diagnostic tools performed by ELISA and lateral flow assay (LFA) using nasopharyngeal and oropharyngeal samples and mAbs to target SARS-CoV-2 antigens. However, there is concern about their sensitivity.<sup>88,89</sup> One study found that a rapid SARS-CoV-2 antigen COVID-19 diagnostic test based on LFA technology had a sensitivity of 82.2% for CT values under 25.<sup>80</sup> Unlike antigen testing that can be used as detection tools for viral infection, serological immunoassays have different roles and serve as screening and surveillance tools during the prevalence of COVID-19.<sup>90</sup> SARS-CoV-2 specific antibodies are usually detected more than 1 week after symptom onset.<sup>91</sup> To recognize these antibodies in serum samples, recombinant viral nucleocapsid or spike (S) proteins, part of them like S1, S2, and RBD, or a mixture, have been immobilized to detect different classes of anti-SARS-CoV-2 antibodies, which include immunoglobulin A (IgA), IgM, IgG, or IgM/IgG in total.<sup>92</sup> Utilization of whole viral antigens for serological methods, due to containing conserved domains, raises the possibility of cross-reactivity with other common circulating CoVs.<sup>93</sup> Protein-based detection of COVID-19 is not limited to manual ELISA. Digital ELISA, CLIA, surface plasmon resonance (SPR),<sup>94</sup> flow cytometry,<sup>95</sup> lateral flow immunoassay (LFIA),<sup>96</sup> and microfiber-based immunoassay<sup>97</sup> are other protein-based tests, which have been used or are in progress for the diagnosis of SARS-CoV-2. Digital ELISA, also called a single-molecule array (Simoa), is a technique whose sensitivity is in the femtomolar range. In this method, the array amounts are almost two billion times lower than a traditional ELISA.<sup>98</sup> In one study, developing a Simoa immunoassay on the automated system to identify SARS-CoV-2 N-protein in venous and capillary blood was reported.<sup>99</sup> In another study, an ultrasensitive digital ELISA based on a Simoa was developed, which is rapid and can simultaneously detect spike and nucleocapsid proteins of SARS-CoV-2.<sup>100</sup> CLIA is a solid-phase immunoassay method in which the label is a luminescent molecule. Other advantages of this method include high signal intensity, absence of interfering emissions, and reduced incubation time.<sup>101</sup> In a study about diagnosing SARS-CoV-2 infection, it was shown that specificity for IgG was greater than 98% for CLIA.<sup>102</sup> SPR is a label-free technique and does not require additional

reagents, assays, or laborious sample preparation steps and is mostly used for large biomolecules. This optical method has been incorporated into immunoassay tools, especially in detecting antibodies.<sup>103,104</sup> In the same context, a sensor detecting nucleocapsid antibodies specific against the COVID-19 was reported, which can use a human serum as a sample.<sup>94</sup> The flow cytometric approach was also validated based on antigen-expressing human embryonic kidney 293 (HEK 293) cells to evaluate spike-specific IgG and IgM antibody reactions. Regarding the results, the specificity and sensitivity were comparable to ELISA or CLIA.<sup>53</sup>

### 3.1.3 | Point of care

Laboratory tests require expensive materials and equipment and take time. In contrast, POC devices are simple, inexpensive, and in-house tests that usually take less than 1 h to respond.<sup>105,106</sup> As of October 12, 2021, the Foundation for Innovative New Diagnostics (FIND), which publishes the lists of all commercially available immunoassays molecular tests for COVID-19, reports that about 402 flow assay strips or cassettes are commercially available.<sup>107</sup> Microfiber-based immunoassay, which is microfiber-based arrays of antigens to capture specific antibodies,<sup>99</sup> and LFIA are some of the POC devices for molecular detection. LFIA is a kind of cellulose-based device and usually employs gold nanoparticles to label antigen or antibody to identify SARS-CoV-2-specific antibody or antigen in specimens.<sup>108</sup> LFIA has also been used for nucleic acid recognition. For instance, the LAMP POC device is used for the fast and on-site recognition of COVID-19, a combination of lateral flow strip and LAMP assay.<sup>106</sup> The other end of care method is RPA with lateral flow dipstick reaction, which is a nucleic acid base method and can be performed in less than 20 min at 10–37°C.<sup>109</sup>

In conclusion, diagnostics are essential for dealing with outbreaks to diagnosis, surveillance, vaccine design, and so on.<sup>110</sup> The congenital COVID-19 diagnostic methods are different in sensitivity, accuracy, specificity, and application. For instance, RT-PCR is a method to detect viral nucleic acid, while serological tests are beneficial for surveillance<sup>111</sup> and vaccine studies.<sup>94</sup> Factors that play a vital role in preventing false negatives in COVID-19 as well as other respiratory diseases include sampling method, sampling time based on symptom onset, and method of sample transmission to the laboratory.<sup>112</sup> The other important parameter is selecting a suitable target based on virology and biology of SARS-CoV-2.<sup>113</sup> Up-to-date, a variety of tests have been commercialized with or without FDA-EUA approval, and others are in research and development.<sup>64</sup>



### 3.2 | X-ray-based detections

Nowadays, RT-PCR is used as an accepted standard method for the identification of COVID-19 infection. However, this laboratory test is expensive and time-consuming. Moreover, the false-negative test result is possible because of insufficient quantities of viral load and incorrect extraction method.<sup>114</sup> Considering all the above disadvantages, we need an accurate, fast, and cost-effective way to detect COVID-19. Chest CT can be an indispensable tool for screening and diagnosing people suspected of COVID-19. In this regard, two common abnormalities in the lung, such as grand-glass opacities and consolidating, are diagnosable by chest CT. The importance of CT is not only limited to detection and diagnosis, but also this test can be used for treatment evaluation and follow-up.

Nevertheless, some studies reported that patients with a severe form of COVID-19 have positive RT-PCR results with standard CT. One possible explanation for this phenomenon is that SARS-CoV-2 targets multiple organs, such as the heart, kidney, and liver, so comprehensive examinations should be performed for better understanding.<sup>114,115</sup> Based on a meta-analysis, the sensitivity of chest CT was great in Wuhan (96–99%) and in other regions of the world was varied from 61% to 98%.<sup>116</sup>

## 4 | PRECLINICAL STUDIES

Cell lines and organoids are a rapid system for studying the interactions and infection processes of the virus with limitations in understanding pathology, antigenic drift, and virus evolution in this system. So, animal models provide the advantage of studying virus replication according to the physiological symptoms of an organism.<sup>117</sup> Despite FDA regulations' flexibility in saving time, in research on SARS-CoV-2 vaccines and antiviral drugs, animal models will play a critical role in preclinical studies. Animal models of nonhuman primates, including African green monkeys, rhesus monkeys, and cynomolgus monkeys, can infect via SARS-CoV-2 with severe lesions and histopathological changes in vital organs. The rhesus monkey is more sensitive, and the African green monkey has more respiratory-related symptoms.<sup>118,119</sup> The similarity of the ACE2 receptor in rhesus macaques, Syrian hamsters, and common marmosets is very high and reaches 100% in rhesus macaques.<sup>118</sup> The hamster ACE2 has the highest affinity for SARS-CoV-2 spike proteins. The viral load in this animal increases, leading to diffuse alveolar damage in the early stages and apoptosis in the later stages of infection. Ferret is more susceptible to the virus than cats, with differences in only two amino acids in ACE2.<sup>120</sup> Because the virus affects the ferret's upper respiratory

tract, they are a potential animal model for evaluating vaccines efficacy.<sup>121,122</sup> Owing to mice's advantages, such as small size and availability, these animals are suitable models for preclinical researches. But mouse ACE2 receptor has less similarity to human ACE2 and low binding affinity for entering the SARS-CoV-2 viruses via their spike protein. Therefore, for studying the transmission and pathogenesis of the virus, evaluating antiviral drugs and vaccine developments, it is necessary to produce mouse-adapted SARS-CoV-2 strains to develop mild to severe disease suitable for studies.<sup>123</sup> There are two pathways to the manipulation of mice for SARS-CoV-2 studies listed in Tables 2 and 3. The first strategy is producing human angiotensin converting enzyme 2 (hACE2) mice to develop vaccines and other potential antiviral therapies, and the second knockout of other genes to mimic disease.

## 5 | CLINICAL INFORMATION

Various sampling paths have been introduced for RT-PCR, such as pharyngeal swab specimens, oropharyngeal swab specimens, urine, and stool samples.<sup>137,138</sup> Fecal and urine sampling is easier than nasopharyngeal swabs and sputum specimens, while the quantity/quality of the samples are simply determined and increase the diagnosis of asymptomatic patients.<sup>139</sup> The ordinary COVID-19 disease symptoms are fever ( $>37.8^{\circ}\text{C}$ ), cough, asthenia, and dyspnea,<sup>140,141</sup> but patients with positive stool tests did not suffer gastrointestinal issues and had nothing to do with the severity of the lung infection<sup>138</sup> and some people have no symptoms.<sup>141</sup> Altered mental status, myocardial, hepatic, and kidney injuries have been reported not only among older patients but also in younger patients.<sup>142</sup> The nonpersistent cough, hoarse virus voice, nausea and vomiting, shortness of breath, nasal discharge or congestion, headache, wheeze, muscle aches, diarrhea, and loss of sense of taste/ smell have also been reported in some cases.<sup>141</sup> The main signs of the onset of the disease in children include abdominal pain, vomiting, and headache.<sup>143,144</sup> A study showed that in some patients with CT scan confirmed COVID-19, the PCR test was negative.<sup>145</sup> In contrast, some COVID-19 positive patients do not represent any of the symptoms mentioned above, and the development of new symptoms makes the diagnosis more complicated.<sup>146</sup>

### 5.1 | Abdominal symptoms

A 55-year-old man was hospitalized with abdominal ache in the left iliac fossa without the usual signs of COVID-19, and no significant changes in the respiratory system were CT-scanned. In evaluating the thoracoabdominal

**TABLE 2** Manipulation of mice for SARS-CoV-2 studies: producing hACE2 mice for the development of vaccines and other potential antiviral therapies

| Mouse model           | Identification   | Utilities   | Benefits   | Limitations   | References |
|-----------------------|--|---|--|---|------------|
| hACE2 mice            | Adenovirus serotype 5 (Ad5)-hACE2-transduced mice                          | Useful for the assessment of SARS-CoV-2-specific therapies, such as vaccine evaluation, human convalescent plasma therapy, and antiviral therapies  | Rapid and efficient mouse lungs expression, and manufacture of an easily reproducible murine model for SARS-CoV-2 within 2–3 weeks   | Severe and extrapulmonary manifestations of the disease                   | 124        |
| hACE2 knockin mice    | ACE2 humanized mouse by CRISPR/Cas9 knockin method                         | Evaluation of the potential therapeutics<br>Development of vaccines; elucidation of the transmission and pathogenesis<br>Validation of the risk factors dependent on the intense symptoms in COVID-19 | In the mice susceptible to SARS-CoV-2 contamination upon intranasal inoculation, hACE2 antigen expression was discovered in the kidneys, vascular endothelium, skeletal muscle, lungs, adrenal, liver, pancreas gastrointestinal, heart, spleen, LN, smooth muscle, and ganglia, and as a consequence, pulmonary contamination and pathological variations be similar to COVID-19 patients | Requires longer periods of time to cross or genetically modify these mice | 125        |
| hACE2-transgenic mice | pCAGGS-ACE2 plasmid with CAG promoter (AC70, AC22, and AC63 mouse lineage) |   |  |   | 126,127    |
|                       | pK18-hACE plasmid with human K18 promoter                                  |   |  |   | 128        |
|                       | pEGFP-N1ACE2 plasmid with mouse ACE2 promoter                              |   |  |   | 129        |
|                       | HFH4-ACE2 plasmid with human HFH4 promoter                                 |   |  |   | 130        |

**TABLE 3** Manipulation of mice for SARS-CoV-2 studies: knockout of other genes to mimic disease

|               | Mouse model           | Utilities   | Benefits   | References |
|---------------|-----------------------|---|--|------------|
| Knockout mice | ACE2 knockout mice    | Model for coronavirus-induced cytokine storm-driven inflammation, etiology, and treatment | Stimulation of acute respiratory distress syndrome (ARDS)      | 131,132    |
|               | Tmprss2 knockout mice | COVID-19 disease pathogenesis   | Development of pneumonitis to accompany with viral replication | 133,134    |
|               | Stat1 knockout mice   | Model for morbidity, viral replication, and mortality                                     | Antivirals and pathogenesis studies                            | 135,136    |

transition pictures, separate ground-glass opacities were discovered on the periphery of the middle lobe and posterior basal segment of the right lobe. A full chest CT was taken for better assessment of degree of pulmonary involvement in which a viral infectious process was found similar to the documents representing pulmonary involvement in COVID-19. This finding was reconfirmed by real-time PCR through the nasal section and oropharynx swab. In another case, an 84-year-old man was referred to the hospital with abdominal pain, 6 days of fever 38°C, mild diarrhea without vomiting, nausea, or abdominal symptoms. A chest CT revealed multiple ground-glass and crazy-paving pulmonary opacities in multifocal, predominantly peripheral, bilateral, and posterior distribution, mostly in the lower lobes, which confirms the COVID-19 infection with certainty.<sup>147</sup> In this case, COVID-19 PCR on oropharynx swab was positive, and laboratory tests demonstrated increased inflammatory serum biomarkers with normal blood cell counts. Patients who were followed-up for autoimmune liver disease, cirrhosis, chemotherapy for hepatoblastoma, and transplantation, none developed a pulmonary disease appeared to be the major driver of the lung tissue injury through this infection.<sup>148,149</sup>

## 5.2 | Hypertension

Although 30% of hospitalized COVID-19 patients have shown hypertension, it should be considered that the hypertension occurrence among the age-matched healthy population is within the same range. Therefore, the hypothesis in which hypertension (and its therapy with ACE2 inhibitors/ARB) rises the danger of severe COVID-19 infection is not supported by these findings. It is best not to increase the dose of angiotensin receptor blocker (ARB) drugs in SARS-CoV-2-infected patients and not to start a new ARB therapy.<sup>150,151</sup>

## 5.3 | Diabetes

Published data suggest that the incidence of diabetes mellitus (DM) in patients with COVID-19 is higher than normal.<sup>152–160</sup> Diabetes can be assumed as an independent

risk factor for increased ICU admissions, need for ventilation, and eventually death.<sup>161,162</sup> It can be suggested that diabetic patients are at a significantly enhanced risk of SARS-CoV-2 and COVID-19. Possibly, enhanced ACE2 expression in DM patients contributes to enhanced sensitivity toward these infections.<sup>163</sup> Also, relatively high ACE2 expression in the pancreas islets may contribute in hyperglycemia among COVID-19 patients.<sup>164</sup> An asymptomatic girl was admitted to the hospital without clinical symptoms. The throat swab was COVID-19 negative by RT-PCR, while the urine test was positive. After antiviral and symptomatic supportive treatments, the throat swab became positive. During the second and third weeks, both tests became negative, while 1 month later, the patient felt well, and throat swab RT-PCR was negative.<sup>139</sup>

## 5.4 | Renal dysfunction

Podocytes and proximal convoluted tubules, as potential host cells for SARS-CoV-2, show considerable expression of coexpression of ACE2 and TMPRSS genes. Severe renal malfunction is one of the fatal complications in COVID-19 patients. Pathophysiological research has shown that this complication resulted by the virus can cause cytopathic effects.<sup>165,166</sup> Reports indicate that 0.5–19% of COVID-19 patients develop acute kidney injury. In studies related to COVID-19, contrast-enhanced imaging (CT and MRI) should be used with more caution, as impaired renal function enhances patients' sensitivity to contrast-induced nephropathy.<sup>167,168</sup>

## 5.5 | Cardiac manifestation

ACE1 is a transmembrane aminopeptidase that is a target receptor for SARS-CoV-2. It is implicated in the development of hypertension and significantly expressed in the heart. Accordingly, the probability of cardiovascular damage/myocarditis is also believed as a symptom of COVID-19. Furthermore, one clinical study reported five confirmed cases of SARS-CoV-2 that showed advanced

myocardial damage through infection. Myocardial injuries are mostly represented by enhanced levels of biochemical markers, containing cardiac creatine kinase, troponin I, lactate dehydrogenase, and  $\alpha$ -hydroxybutyrate dehydrogenase.<sup>168–170</sup>

## 5.6 | Mediastinal findings

Mediastinal lymphadenopathy has been observed in COVID-19 patients.<sup>171</sup> A recently published article reported enlarged mediastinal lymph node as a common symptom in patients with severe COVID-19. Therefore, lymphadenopathy is another symptom of COVID-19, especially in acute patients.<sup>172,173</sup>

## 5.7 | Neurological discoveries

Corona family viruses can go into the central nervous system (CNS) via the neuronal retrograde route or bloodstream heading to encephalitis or meningitis with mortality and morbidity. While viral encephalitis can continue undiagnosed because of symptoms absence, acute viral encephalitis can affect body temperament, mental status, abnormal motor movement, irregular behavior/speech, and focal neurological irregularities as flaccid paralysis, hemiparesis, paresthesia, or seizures.<sup>174</sup> SARS-CoV-2 can find its path via the circulation or through the cribriform plate of the ethmoid bone and attack the CNS. It can also interact with ACE2 receptors and damage nerve tissues. COVID-19 cerebral attachment via the cribriform plate may cause further complications, such as hyposmia/anosmia.<sup>175–177</sup>

## 5.8 | Hematological symptoms

Pathological studies on SARS-CoV-2 pathogenesis derived that the COVID-19 disease was instead a hypersensitivity pneumonitis than viral pneumonia.<sup>178</sup> SARS-CoV-2 stimulates a cytokine storm (hyperactive immune response) and spilled high levels of cytokines into the circulatory system, which leads to systemic issues across multiple organs. SARS-CoV-2 pneumonia can cause multiorgan failure by overproduction of proinflammatory cytokines combine with a diminished oxygenation capacity of the patient's blood. Other symptoms of severe cases contain septic shock, difficult-to-correct metabolic acidosis, and coagulation dysfunction.<sup>179–183</sup>

## 5.9 | COVID-19 and pregnancy

Recent studies on pregnant women infected with COVID-19 have shown that the main symptoms of their clinical manifestations include fever and cough and are no differ-

ent from those of nonpregnant adults. Also shown that pregnant women are more prone to COVID-19 and its complications and may even become severely ill.<sup>184</sup> Even though there is no significant evidence to confirm vertical transmission, the possibility of mother-to-child transmission of COVID-19 or SARS infection is not ruled out.<sup>185–189</sup>

## 5.10 | Autoimmune diseases

Through an examination of patients who suffer from autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, and Sjogren's syndrome, it was shown that anti-SARS-CoV-2 IgG and IgM antibodies were not detected in serum samples, indicating that there was no cross reactivity between autoantibodies and SARS-CoV-2 antibodies.<sup>190,191</sup>

## 5.11 | Cancer

The results of a study conducted on cancer patients revealed that these patients might be more susceptible to infection in comparison to noncancer subjects. The rate of COVID-19 mortality was higher in cancer patients showing worse prognoses for older ages and women. Combination therapy by antiviral medicines plus hydroxychloroquine (HCQ) seems superior to HCQ alone.<sup>192</sup> In another study, the infection caused severe clinical events in Chinese cancer patients.<sup>193</sup> It was also reported that 10.7% of virus-positive cancer patients had symptoms.<sup>194,195</sup>

## 6 | TREATMENT

There is still no cure for COVID-19. However, only one treatment, a drug called remdesivir, has been approved by the FDA for this disease, and research suggests that it provides only modest benefit to patients.<sup>196</sup> Therefore, the best first-line actions against this disease are staying home, washing hands, wearing the face mask, and having adequate rest. In this review, we summarized some potential treatments against this new emerging virus.

### 6.1 | Therapeutic agents and inhibitors against COVID-19

Here, we aim to present some of the potential and repurposed drugs for the treatment of COVID-19.

#### 6.1.1 | Antiviral drugs

The most important antiviral drugs used to treat COVID-19 virus are listed in Figure 3 and are discussed in detail below.

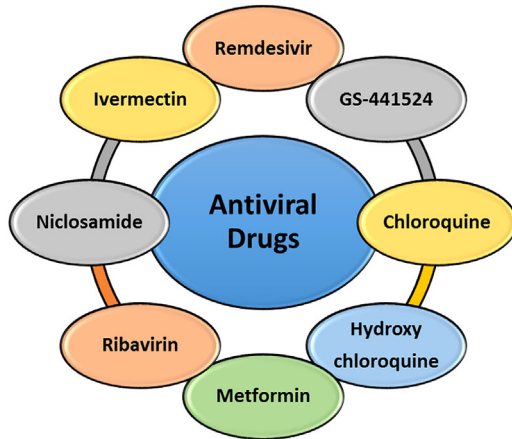


FIGURE 3 Antiviral drugs against COVID-19

### Remdesivir

Remdesivir (GS-5734), a broad-spectrum antiviral agent, was synthesized and developed in 2017. It is a monophosphate prodrug, which belongs to the class of nucleotide analogs (Table S1). Owing to its low half maximal effective concentration ( $EC_{50}$ ) and host polymerase selectivity against the Ebola virus, it was used to treat the Ebola virus disease. This drug is adenosine analog and decreases viral RNA production by obscuring viral RNA polymerase and evading proofreading by viral exonuclease.<sup>197</sup> Moreover, further research revealed considerable antiviral activity against MERS-CoV and SARS-CoV viruses, particularly human coronavirus 229E.<sup>198–202</sup> As an only drug approved by the FDA for the treatment of SARS-CoV-2, it is currently being monitored in multisite clinical trials. The evidence of the antiviral effects of GS-5734 on coronaviruses in vitro and in vivo studies have demonstrated favorable efficacy and low toxic side effects.<sup>198,199,203,204</sup> One survey showed that the combination of remdesivir and emetine has a synergy effect and inhibits SARS-CoV-2 replication in vitro.<sup>205</sup> Moreover, the efficacy of remdesivir and chloroquine (CQ) in the inhibition of COVID-19 was demonstrated.<sup>206</sup> Also, the U.S. FDA has issued an emergency license for remdesivir to expedite the treatment of SARS-CoV-2.<sup>196</sup>

### GS-441524

GS-441524 is an antiviral drug that is the parent nucleoside of remdesivir (Table S1). It has been found that GS-441524 exhibits antiviral activity on SARS coronavirus (SARS-CoV), Marburg virus, and feline infectious peritonitis virus. Several in vitro and in vivo studies on this drug against COVID-19 using a mouse and non-human primate animal models have reported favorable outcomes.<sup>197,203</sup> Nevertheless, another investigation based on a more comprehensive pharmacokinetic rationale supported the application of GS-441524 over remdesivir for the treatment of SARS-CoV-2 due to its synthetic simplicity and in vivo efficacy in the veterinary setting.<sup>202</sup>

### Chloroquine

CQ is an antiviral drug that has been long employed in the prevention and treatment of malaria and also exhibited in vitro activity against the replication of some coronaviruses, such as the SARS-CoV-2 (Table S1).<sup>197,207,208</sup> The safety and tolerability of this drug have been approved previously.<sup>209</sup> It was found that CQ inhibits the glycosylation of host receptors, proteolytic processing, and endosomal acidification, which results in blocking viruses from entering into cells. Furthermore, it modulates immunity by attenuating cytokine production and the inhibition of autophagy and lysosomal activity in host cells.<sup>210,211</sup> Consequently, this molecule was suggested as a potential drug in the treatment of COVID-19.<sup>212</sup> However, this drug's efficacy (with or without a macrolide) in patients with COVID-19 was not confirmed by available clinical data, although an increased frequency of ventricular arrhythmias and decreased in-hospital survival were observed.<sup>213,214</sup> According to the latest update of the Coronavirus Drug and Treatment Tracker (October 7, 2021), this drug is not considered promising, meaning early evidence suggests that these treatments do not work.<sup>215</sup>

### Hydroxychloroquine

HCQ is an antiviral and antimalarial drug, a safer, less toxic, and more potent derivative of CQ. It is currently widely used to treat autoimmune diseases, rheumatoid arthritis, systemic lupus erythematosus, sarcoidosis, alopecia areata, and antiphospholipid syndrome (Table S1).<sup>207,211</sup> This drug directly inhibits viral entry into cells with the same mechanisms of action for CQ. So, it has been proposed as an antiviral drug in the treatment of several coronaviruses, especially SARS-CoV-2.<sup>198,199,211,216</sup> In preliminary clinical trials, the clinical efficacy of HCQ in combination with azithromycin in vivo was reported.<sup>217,218</sup> On the other hand, the use of HCQ (with or without a macrolide) in patients hospitalized with COVID-19 was not supported by other studies, and adverse side effects were also found in the patients.<sup>214,215,219–221</sup> An open-label non-randomized clinical trial demonstrated that HCQ reduced viral load in COVID-19 patients, and azithromycin reinforced its effect.<sup>222</sup> Finally, the FDA warns that the drug can have several serious side effects on the heart and other organs when used to treat COVID-19. On March 2, a WHO expert panel strongly advised against the use of HCQ in all patients, adding that the drug was no longer a research priority.<sup>215</sup>

### Metformin

Metformin was initially announced as an anti-influenza medication that glucose-lowering was known as one of its side effects. This drug has pleiotropic effects and may be

effective against hepatitis C virus (HCV), hepatitis B virus, and human immunodeficiency virus (HIV). At the molecular level, metformin phosphorylates AMP-activated protein kinase (AMPK) in hepatocytes and causes its activation. The SARS-CoV-2 binds to the ACE2 receptor to enter the host cell using RBD in the spike protein. It has been hypothesized that ACE2 causes acute lung injury through the AMPK pathway and autophagy. Therefore, the phosphorylation of ACE2 can change its conformation and function and reduce virus entry.<sup>223</sup> In this regard, a retrospective analysis demonstrated that metformin treatment decreased the mortality rate in COVID-19 patients with diabetes.<sup>224</sup>

#### *Ribavirin*

Ribavirin, a guanosine analog, has antiviral activity, so it is recommended for COVID-19 treatment. The efficacy of the combination of ribavirin with IFN alfa in virus clearance and survival of patients was reported in the experience of MERS-CoV. However, no significant benefit was observed with ribavirin treatment compared to the control group in hospitalized patients with severe or critical COVID-19.<sup>198,199,225</sup>

#### *Niclosamide*

Niclosamide, an FDA-approved anthelmintic drug, has various antiviral activities. The efficacy of this drug against SARS-CoV, MERS-CoV, ZIKV, and HCV has been investigated. The potential antiviral mechanism of niclosamide against SARS-CoV-2 is inhibition of autophagy, viral application, and receptor-mediated endocytosis.<sup>226,227</sup> The clinical efficacy of niclosamide against COVID-19 should be evaluated.

#### *Ivermectin*

Ivermectin is a wide-ranging antiparasitic that was approved by the FDA (Table S1). It has been reported to exhibit in vitro efficacy against several viruses, such as dengue virus, Zika virus, yellow fever virus, SARS-CoV-2, and so on. However, this drug has some side effects, such as neurotoxicity.<sup>228</sup> The single addition of this inhibitor of COVID-19 to Vero-hSLAM cells led to over 5000-fold reduction in viral RNA within 48 h. Nonetheless, an increase in time, that is, up to 72 h, did not result in more viral replication reduction. Finally, a trial on 1500 patients found no benefit from ivermectin.<sup>215,229</sup>

### 6.1.2 | Calcineurin inhibitors

#### *Cyclosporine (cyclosporin)*

Cyclosporine (cyclosporin) is a calcineurin inhibitor and belongs to immunosuppressant drugs used to prevent rejection after organ transplantation (Table S1). Since it

binds to cyclophilins in cells and consequently inhibits replicating different coronaviruses, it has been proposed to use against COVID-19.<sup>230,231</sup> Nevertheless, clinical trials should be considered in further studies.

### 6.1.3 | Cyclophilin inhibitors

#### *Alisporivir*

Alisporivir is a nonimmunosuppressive cyclophilin inhibitor, which is derived from cyclosporine (Table S1). This inhibitor blocks the replication of four different coronaviruses, such as SARS and MERS.<sup>232</sup> It also inhibits in vitro SARS-CoV-2; thus, it has been recommended as a potential candidate for the treatment of COVID-19.<sup>233</sup>

### 6.1.4 | HCV inhibitors

#### *Sofosbuvir*

Sofosbuvir is a clinically approved drug against the HCV (Table S1). The high sequence and structural homology in the RdRp of the HCV and SARS-CoV-2 viruses support the idea that sofosbuvir can effectively inhibit the SARS-CoV-2 RdRp. Moreover, the safety profile of this drug has been known due to its long-lasting application in patients for treating and eradicating HCV chronic infection. As a result, it has been suggested to use sofosbuvir to treat SARS-CoV-2.<sup>234,235</sup>

### 6.1.5 | HIV protease inhibitors

#### *Lopinavir/ritonavir (LPV/r)*

Lopinavir (LPV) is an antiretroviral that inhibits the activity of the protease. Also, ritonavir (RTV) is another antiretroviral that belongs to the protease inhibitor class (Table S1). RTV helps to stabilize LPV through an increase in its plasma half-life. The combination of these agents results in lopinavir/ritonavir (LPV/r) approved by the U.S. FDA and is used to treat HIV. It has also been shown to have in vitro and in vivo activities on other new coronaviruses, such as SARS and MERS, by inhibiting the 3-chymotrypsin-like protease (3CL<sup>PRO</sup>), also known as main protease (M<sup>PRO</sup>).<sup>198,200,236,237</sup> However, the use of LPV/r for the treatment of COVID-19 was not supported.<sup>198,199,219,238</sup>

In the case of MERS-CoV, the efficacy of a combination of LPV-RTV with ribavirin and IFN alfa has been studied. However, a trial study of LPV/RTV in adults hospitalized with severe COVID-19 showed that there is no benefit with LPV-RTV treatment beyond standard care.<sup>239</sup>

#### *Nelfinavir*

Nelfinavir is another HIV-1 protease inhibitor that reduced the replication of SARS-CoV-2 in vitro. So, it can be considered as a potential drug in the treatment of

COVID-19.<sup>240</sup> Moreover, this drug may inhibit SARS-CoV-2 spike-mediated cell fusion.<sup>241</sup> One study demonstrated the high potency of nelfinavir against SARS-CoV-2 in Vero E6 cells. However, further exploration as a potential treatment for COVID-19 is needed.<sup>242</sup>

#### *Darunavir/cobicistat*

This combination can be a potential alternative to LPV/RTV based on a similar mechanism of action. However, a single-center, randomized, and open-label trial on mild patients with confirmed COVID-19 demonstrated that this HIV-1 protease inhibitor might not have clinically significant anti-SARS-CoV-2 activity. As a result, more studies are needed to investigate its role.<sup>225</sup>

### 6.1.6 | Anti-influenza drugs

#### *Favipiravir*

Favipiravir is a broad-spectrum antiviral drug (Table S1), a prodrug of a purine nucleotide. It was used against new influenza in Japan. This drug appears to have a role as an inhibitor of the RNA-dependent RNA polymerase, which leads to stopping viral replication. It exhibited good efficacy in the treatment of influenza and Ebola virus in pre-clinical studies and showed activity against other RNA viruses. Regarding the in vitro activity of favipiravir against SARS-CoV-2 with the EC<sub>50</sub> value of 61.88 μM/L in Vero E6 cells and relatively safe use of this agent, in addition to its benefit in early clinical trials, the application of favipiravir for the treatment of COVID-19 has been recommended. However, more research is needed.<sup>199,200,243,244</sup> Studies have shown that this drug is effective in disease progression and viral clearance.<sup>245</sup> Recently, a review of favipiravir trials found that this drug has a negligible effect on mortality in patients with severe symptoms.<sup>246,247</sup>

#### *Arbidol*

Arbidol is another anti-influenza drug, and it is a membrane fusion inhibitor. This drug has been approved in Russia and China for treating infections associated with influenza A and B and other arboviruses. Arbidol is prescribed for adults with COVID-19 disease.<sup>248,249</sup> One study illustrated that COVID-19 treatment with Arbidol monotherapy is superior to LPV/RTV.<sup>250</sup>

#### *Oseltamivir*

Oseltamivir was approved to use against influenza A and influenza B. This drug targets neuraminidase, which is distributed on the surface of the virus. Oseltamivir is under investigation in clinical trials to treat COVID-19 in combination with other drugs, such as CQ and favipiravir.<sup>237</sup>

### 6.1.7 | Antibiotics

#### *Azithromycin*

Azithromycin is an antibiotic that belongs to macrolides (Table S1). It is employed to treat a large variety of bacterial infections, such as bronchitis, pneumonia, and *Mycobacterium avium* complex infection. It regulated the pH of endosomes and trans-Golgi network by acting as an acidotropic lipophilic weak base.<sup>215</sup> It has also been effective against SARS-CoV-2 when combined with HCQ in preliminary clinical trials.<sup>200,216,177,251</sup> However, a large-scale randomized clinical trial found no benefit of azithromycin in patients hospitalized with COVID-19.<sup>252</sup>

### 6.1.8 | Other drugs for COVID-19

Other therapeutic agents that have been proposed for the treatment of COVID-19 include copper,<sup>253</sup> nasal nitric oxide,<sup>254</sup> Qingwen Baidu Decoction,<sup>255</sup> sodium chromo-glycate and palmitoylethanolamide,<sup>256</sup> bovine lactoferrin,<sup>257</sup> α-ketoamides,<sup>258</sup> mammalian target of rapamycin inhibitors,<sup>259</sup> 1-thia-4-azaspiro[4.5]decan-3-one derivatives,<sup>260</sup> H<sub>2</sub>S-producing compounds,<sup>261</sup> Gemcitabine, lycorine, and oxysophoridine,<sup>262</sup> Atovaquone, Mebendazole, and Ouabain,<sup>263</sup> and bruton's tyrosine kinase inhibitors.<sup>264</sup> These possible drugs need further clinical trials. On the other hand, the auranofin,<sup>265</sup> eukaryotic initiation factor 4A inhibitor silvestrol,<sup>266</sup> Liu Shen capsule,<sup>267</sup> methylprednisolone,<sup>268</sup> tilorone,<sup>269</sup> HTCC,<sup>270</sup> indomethacin and resveratrol,<sup>271</sup> and naproxen<sup>272</sup> compounds have been tested, some of which showed good efficacy against SARS-CoV-2. However, more comprehensive studies on these drugs should be considered.

## 6.2 | Supporting agents

### 6.2.1 | Vitamins

#### *Vitamin C*

The beneficial effects of vitamin C in improving common colds and pneumonia have been shown previously. Vitamin C decreases the mRNA expression of proinflammatory cytokines in obese patients in vitro. Moreover, the combination of C and E vitamins decreases oxidative stress and reduces the viral load in patients with HIV infection. Furthermore, vitamin C can stimulate the intracellular type I IFN system, which performs the antiviral activity.<sup>273</sup>

#### *Vitamin D*

This vitamin reduces the risk of viral infection and mortality using different mechanisms, such as decreasing the

cytokine storm, regulating adaptive immunity, and stimulating T cell induction. Therefore, it is better for people who are at high risk for COVID-19 infection to maintain vitamin D at the optimal level in their circulating blood.<sup>273,274</sup>

#### *Vitamin E and A*

Vitamin E has some acute effects on the immune system, such as improving the activation of natural-killer, naive T-lymphocytes, and DC, and inhibiting the production of proinflammatory cytokines, including IL-1, IL-6, and TNF. Vitamin A and its metabolites can regulate the innate and adaptive immune system and modulate cytokine production, differentiation, and so on.<sup>274</sup>

#### *Convalescent plasma treatment*

According to two systematic review studies, convalescent plasma treatment could significantly reduce the viral load and increase the level of neutralizing antibodies in infected patients. Moreover, the general condition of all patients improved after convalescent plasma transfusion. On March 24th, 2020, the FDA approved convalescent plasma transfusion as a treatment option for patients in life-threatening conditions. However, this treatment strategy has some limitations, such as increased thrombotic event risk and finding volunteer donors with high neutralizing antibody titers, and the optimal dosage.<sup>275,276</sup> Finally, the Infectious Disease Society of America discourages the use of convalescent plasma in hospitals, saying that there is no evidence yet to support its use in people in the early stages of their infection.<sup>215</sup>

#### *Melatonin*

Melatonin is a hormone that helps to adjust the cycle of body's sleep-wake. It is produced in the pineal gland. Melatonin secretion gradually decreases with age. It is thought that this hormone, with its immune-modulatory and antiviral properties, can be useful as a prophylactic treatment against COVID-19. However, using this prophylactic treatment may have side effects, such as dizziness, headache, nausea, and sleepiness.<sup>277</sup>

#### *Monoclonal antibodies*

The efficacy of mAbs, as a promising class of drugs, has been shown against some viral infectious diseases. The target of the mAb is vulnerable sites of viral surface proteins. For example, the human 47D11 mAb targets the full-length spike proteins of SARS-CoV and SARS-CoV-2 and may be a potential option for the prevention and treatment of COVID-19. It was suggested that the combination of mAbs and the remdesivir could be an ideal treatment strategy.<sup>278</sup> Nowadays, mAbs are categorized as widely used treatment against COVID-19. For example, Regneron won an emergency use authorization for REGEN-COV in November.

The FDA authorized its use for patients with mild to moderate cases who are at high risk of progressing to severe COVID-19.<sup>279</sup>

*Tocilizumab.* Tocilizumab is a humanized mAb IL-6 receptor antagonist with the protein chemical formula of  $C_{6428}H_{9976}N_{1720}O_{2018}S_{42}$  that is used to treat rheumatoid arthritis and cytokine release syndrome, a side effect of chimeric antigen receptor T cells anticancer therapy.<sup>199,200</sup> Therefore, it has been used in some severe cases of COVID-19, which led to early good results.<sup>199,280,281</sup> However, another study recommended to use this drug cautiously in severe and critical COVID-19 cases and did not support the evidence of clinical improvement.<sup>282</sup> However, WHO recommended this drug for patients with severe or critical COVID-19 infection.<sup>215</sup>

#### *Interferons*

The efficacy of IFN therapy was investigated in the SARS-CoV and MERS-CoV pandemic. A multicenter randomized open-label phase 2 trial in COVID-19 revealed that a multiple combination of an injectable of IFN beta-1b with LPV-RTV and ribavirin could reduce virus shedding and hospital stay.<sup>283,284</sup> However, Su and Jiang declared that the role of IFN in the pathogenesis of SARS-CoV-2 is suspicious and may enhance the expression of the ACE2 receptor.<sup>285</sup> On July 20, the British pharmaceutical company Synairgen announced that an inhaled form of IFN called SNG001 reduced the risk of severe Covid 19 infection in infected patients in a small clinical trial.<sup>286</sup>

#### *Corticosteroid*

Corticosteroid has two main classes: glucocorticoids and mineralocorticoids. Glucocorticoids reduce inflammation in COVID-19 patients. The WHO recommends not to use glucocorticoids for COVID-19 treatment unless for patients with the acute respiratory syndrome. Dexamethasone is a derivative of glucocorticoids. Recent studies showed that Dexamethasone reduces the risk of death in receiving ventilation and requiring oxygen patients. However, this drug does not have a benefit for patients not requiring respiratory support.<sup>287</sup> The British government estimated that the drug had saved one million lives worldwide.<sup>215</sup> According to a study, Tocilizumab, another inflammatory agent, is useful in the case of severe and critical COVID-19 patients and reduced the mortality rate.<sup>288</sup>

## 6.3 | Other potential therapies

### 6.3.1 | Aptamer-based therapy

In recent years, aptamer has attracted the attention of scientists as an alternative to antibiotics. These



oligonucleotides (RNA, ssDNA, and peptide molecules) have their specific 3D structure and connect to their targets with high affinity and sensitivity. Since coronaviruses and orthomyxoviridae (influenza viruses) are RNA viruses with a similar infection mode, aptamers designed for influenza viruses can be investigated for SARS-CoV-2 therapy. A22 is a DNA-type aptamer against influenza H5N1. This aptamer reduced the viral load in BALB/c mice up to 90%. Another example of DNA-type aptamer is C7-35M against influenza H9N2. This aptamer inhibited viral infections in a dose-dependent manner.<sup>289</sup>

### 6.3.2 | Chinese folk medicine

From the previous coronavirus outbreaks, it was approved that some Chinese folk medicine has antiviral activity, so therapies used in previous epidemics can help to control the disease caused by SARS-CoV-2 and be a starting point for new treatments. Helicase can be considered as a drug target because they are necessary for viral replication. Myricetin, scutellarein, and baicalein are suitable substances that can inhibit the hydrolysis of SARS-CoV-1 nsP13. Moreover, it was demonstrated that the biflavonoid and amentoflavone were effective against MERS-CoV helicase nsP13.<sup>290</sup>

Glycyrrhizin (GL), a triterpene, has various biological functions and can be considered as an antiviral drug for the treatment of COVID-19. It is assumed that this drug has some antiviral effects and inhibits virus binding to the ACE2 receptor, downregulates proinflammatory cytokines expression, and induces endogenous IFN production.<sup>291</sup> GL is a frequent component in Chinese folk medicine.<sup>292</sup>

### 6.3.3 | Mesenchymal stem cell therapy

Mesenchymal stem cell (MSC) therapy is broadly used in treating spinal cord injury, type 2 diabetes, autoimmune disease, and some other diseases. Recently, the use of MSCs in the clinical treatment of H5N1 viral infections has also been suggested. MSCs using their immunomodulatory effect to protect alveolar epithelial cells prevent pulmonary fibrosis and cure lung dysfunction.<sup>293</sup>

### 6.3.4 | Small-interfering RNA

Small-interfering RNA (siRNA) is a class of double-stranded and noncoding RNA molecules. The length of this molecule is 20–25 base pairs. It can regulate the expression of genes and so far was implemented for cancer, virus, and genetic disease therapies. In the experience of SARS

and MERS, siRNA was effectively used. Therefore, this approach may be useful for COVID-19 treatment.<sup>294</sup> However, formulating an effective delivery system is a limitation to this approach.

### 6.3.5 | Bacillus Calmette–Guerin vaccination

It has been described that Bacillus Calmette–Guerin (BCG) vaccination offers broad protection against respiratory infections. It was discovered that countries such as Italy, Netherland, and the United States that do not have universal policies of BCG vaccination have been more severely influenced in comparison with countries that have universal and long-standing BCG policies.<sup>295</sup>

## 6.4 | Vaccine strategy

Designing a vaccine against SARS-CoV-2 is challenging. For example, the mutation rate is high in RNA viruses, so the manufactured vaccine may lose its efficacy rapidly. Moreover, an ideal vaccine should decrease transmission, induce herd immunity and long-lived immunity. In SARS-CoV-2, herd immunity would require vaccination of about 67% of the population. Protein subunits, RNA, DNA, non-replicating vector, replicating vector, inactivated virus, and the attenuated virus are among the main strategies for vaccine design.<sup>296</sup> Up to now, eight vaccine candidates have achieved regulatory authorization or approval around the globe for full use.<sup>215</sup> For better understanding, we classified these vaccine strategies into two major platforms: (1) classic vaccine platforms and (2) next-generation vaccine platforms.

### 6.4.1 | Classic vaccine platforms

We can put virus-based and protein-based vaccines in this category. These classical vaccine platforms were successful in the eradication of some diseases, such as smallpox. However, using these platforms is time-consuming, and the development of a vaccine for this pandemic must be done quickly.<sup>297</sup> A whole-inactivated virus is a kind of virus-based vaccine. This strategy is used against current influenza. This kind of vaccine is safe and easy to prepare.

Moreover, it induced potent serum neutralizing antibodies. However, they may cause disease in highly immunosuppressed individuals. In the category of authorized or approved vaccines, BBIBP-CorV (Chinese company Sinopharm), CoronaVac (Chinese company Sinovac), and Covaxin (produced by Bharat Biotech) used this strategy. These three mentioned vaccines are developed in

**TABLE 4** Different authorized or approved vaccine strategies against SARS-CoV-2

| No. | Brand                 | Type               | Developers  | Origin country    | Approval   |
|-----|-----------------------|--------------------|---|-------------------|--|
| 1   | BNT162b2              | mRNA-based vaccine | Fosun Pharma, Pfizer, and BioNTech                    | Multinational     | Approved in the United States and other countries<br>Emergency use in E.U. and other countries |
| 2   | mRNA-1273 or Spikevax | mRNA-based vaccine | Moderna   | The United States | Approved in Switzerland<br>Emergency use in the United States, E.U., other countries           |
| 3   | Sputnik V             | Ad26, Ad5          | Gamaleya Research Institute                           | Russia            | Emergency use in Russia and other countries  |
| 4   | Vaxzevria             | ChAdOx1            | Oxford-AstraZeneca                                    | British-Swedish   | Approved in Brazil<br>Emergency use in the UK, E.U., and other countries                       |
| 5   | Ad26.COVS.2           | Ad26               | Johnson & Johnson                                     | The United States | Emergency use in the United States, E.U., and other countries                                  |
| 6   | BBIBP-CorV            | Inactivated        | Sinopharm-Wuhan                                       | China             | Approved in China, UAE, and Bahrain<br>Emergency use in other countries                        |
| 7   | CoronaVac             | Inactivated        | Sinovac   | China             | Approved in China<br>Emergency use in other countries  |
| 8   | Covaxin               | Inactivated        | Bharat Biotech  | India             | Emergency use in India and other countries   |
| 9   | BIV1-CovIran          | Inactivated        | Amirabad Virology Lab, Shifa Pharmed Industrial Group | Iran              | Emergency use in Iran  |

China and India (Table 4). The efficacy of BBIBP-CorV vaccine was reported 78.1% and it is approved in China, Bahrain, UAE, and other countries. The efficacy of CoronaVac varies between 50.65% in Brazil trial to 83.5% in Turkey trial. The injection of this vaccine has approved in China and other countries. The efficacy of Covaxin was reported 77.8% and it was the first COVID-19 vaccine developed in India to receive emergency approval.<sup>215</sup> The BIV1-CovIran vaccine is another vaccine that has been developed based on this strategy. Preclinical study has highlighted the BIV1-CovIran vaccine as a potential candidate to induce a strong and potent immune response that may be a promising and feasible vaccine to protect against SARS-CoV-2 infection.<sup>298</sup> Emergency use of this vaccine has been issued in Iran and its immunogenicity has been reported at about 90%.

A live-attenuated virus is another example of classic platforms. The measles, mumps, rubella, and polio vaccines are examples of live-attenuated virus vaccines. The development of this kind of vaccine may be rapid. However, reversion by mutation or recombination is possible.

The SARS-CoV-2 has three surface-exposed proteins: the M protein (membrane protein), the E protein (envelope protein), and the S protein (spike protein). Among them, the S protein is a promising vaccine candidate. Besides full-

length S protein, the S1 domain, the RBD, and NTD can be considered vaccine candidates. Since S protein is conserved in SARS and MERS, designing a subunit vaccine based on this protein can make cross-protection. This strategy has been successful in creating the diphtheria, tetanus, pertussis, and hepatitis B vaccines. This kind of vaccine is safe and induces a cellular and humoral immune response. However, they may be too expensive.<sup>299</sup> In the category of authorized or approved vaccines, EpiVacCorona (Vector Institute) and Novavax are the only protein-based vaccines developed and approved in Russia and the United States, respectively. The efficacy of EpiVacCorona vaccine is unknown (Table 4).<sup>215</sup>

#### 6.4.2 | Next-generation vaccine platforms

Designing these kinds of vaccines is based on sequence information. Viral vector and nucleic acid-based vaccines are putting in this category. The viral vector can be a promising vaccine design strategy because of endogenous antigen production and both humoral and cellular immune response stimulation.<sup>296</sup> Sputnik V (Gamaleya Research Institute) is a nonreplicating viral vector (Ad5 and Ad26) designed in Russia. This vaccine is among

**TABLE 5** The list of vaccine candidates at phase 3 clinical trials

| No. | Brand                       | Type        | Developers       | Origin country    | Approval  |
|-----|-----------------------------|-------------|------------------|-------------------|---|
| 1   | Convidecia                  | Ad5         | CanSino          | China             | Approved in China<br>Emergency use in other countries |
| 2   | EpiVacCorona and Aurora-CoV | Protein     | Vector Institute | Russia            | Approved in Turkmenistan<br>Early use in Russia       |
| 3   | NVX-CoV2373                 | Protein     | Novavax          | The United States | –   |
| 4   | Sinopharm                   | Inactivated | Sinopharm-Wuhan  | China             | Approved in China<br>Limited use in UAE               |

authorized or approved vaccines with the efficacy about 91.6% (Table 4). Vaxzevria (manufactured by the British-Swedish company AstraZeneca) is a vaccine based on ChAdOx1 (chimpanzee adenovirus). The vaccine has an efficacy of 74% against symptomatic COVID-19 and 100% against severe or critical COVID-19. Convidecia (Chinese company CanSino Biologics) used Ad5 to develop vaccine against COVID-19 with the efficacy about 65.28%. Ad26.COVS.2 (Johnson & Johnson's vaccine) makes the third coronavirus vaccine available in the United States. The efficacy of this vaccine is about 72% in the United States, 68% in Brazil, and 64% in South Africa.<sup>215</sup>

DNA and RNA vaccine strategies are safe and easy to develop and manufacture. However, the DNA vaccine strategy has some disadvantages, for example, lower immune response and toxicity. On the other hand, RNA vaccines are unstable under physical conditions.<sup>296</sup> Despite all the mentioned disadvantages, Pfizer-BioNTech is a successful vaccine developer that uses an mRNA-based vaccine strategy. This vaccine is currently injected in the UK, Bahrain, Canada, Mexico, the United States, and other countries with the efficacy of 91% (Table 4). The mRNA-1273 or Spikevax (Boston-based Company Moderna) is the second vaccine that used this strategy. This vaccine prevents COVID-19 illness about 93.2% and prevents severe disease about 98.2%.<sup>300</sup>

In addition to vaccines that have been fully approved or licensed for emergency use (discussed above), a number of other vaccines are undergoing the third phase of clinical trials. These vaccines are summarized in Table 5.<sup>215</sup>

## 7 | NANOTECHNOLOGY AND CORONA VIRUSES

### 7.1 | Application of nanotechnology to confront coronaviruses

Nanotechnology and nanomaterials are proved to prevent the spread of the virus in the environment, improve diagnosis, assist in vaccine formulation, and facilitate targeted delivery of antiviral drugs (Figure 4).<sup>301</sup>

#### 7.1.1 | Prevent the spread of the virus in the environment

One of the most important applications of nanotechnology is to prevent the spread of coronaviruses in the environment outside the body and limit transmission. Transmission of coronaviruses, especially COVID-19, occurs with two main approaches. One is transmission via droplets scattered in the air through the patient's sneezing and coughing, and the other is contact with the virus-infected surfaces.<sup>302</sup> The shelf-life of the virus on contaminated surfaces depends on the type of body, and the shelf-life of the virus in the air is estimated to be 3 h.<sup>303</sup> To prevent the spread of the virus in the air, using face masks for people in public places and using air filters and disinfectants for closed environments such as hospitals are recommended. Depositing a layer of nanomaterials on N95 and FFP3 face masks—which has given them hydrophobicity and self-disinfection properties,<sup>304,305</sup> fabricating a replaceable nanoporous membrane to prevent coronavirus entry for N95 face masks,<sup>306</sup> applying nanocoatings for air filters to create the self-disinfecting property<sup>307,308</sup> and using nanodisinfectants in hospitals environment<sup>309</sup> are examples of the use of nanotechnology to prevent the spread of viruses in the air.

To prevent virus transmission through contaminated surfaces, the nanocoating was designed using metal nanoparticles, such as Ag, Cu, ZnO, and Ti<sub>2</sub>O<sub>3</sub>.<sup>310,311</sup> Nanomaterials can be used in polymer paints and coatings for medical devices, walls, and highly contact surfaces.<sup>312</sup> One of the proposed mechanisms to prevent the spread of the COVID-19 on surfaces through nanocoatings is shown in Figure 5.<sup>309</sup> Silver nanoparticles-based nanodisinfectants are used in hospitals and high-contact surfaces in public places.<sup>311</sup>

Metal nanoparticles, such as metal oxides and graphene, can be used for these applications due to their antiviral, antibacterial, and antifungal properties. According to the high surface-to-volume ratio, small amounts can effectively inactivate the virus.<sup>312</sup> Antiviral and antimicrobial nanomaterials and their mechanisms of micro-organisms inactivation are summarized in Table 6.<sup>313,314</sup>

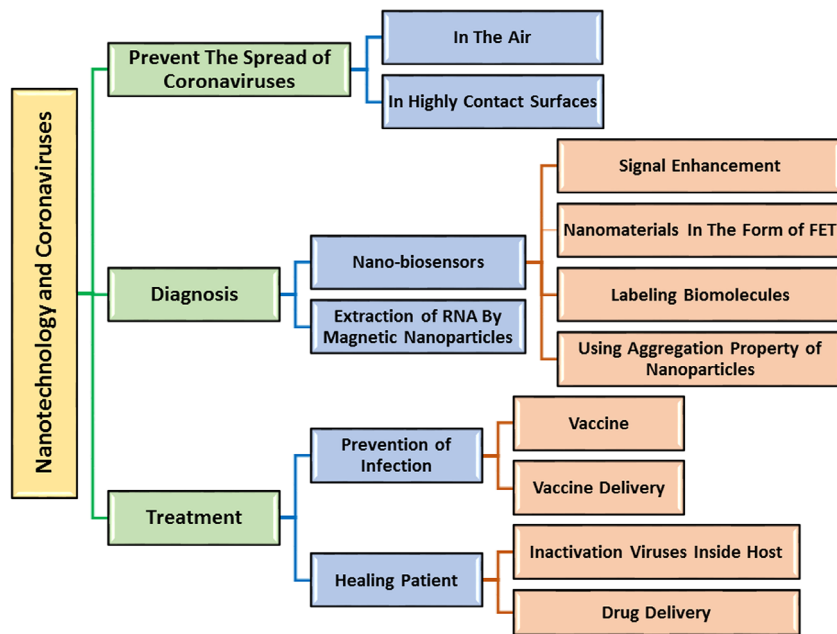


FIGURE 4 Nanotechnology applications in various stages of confronting coronaviruses

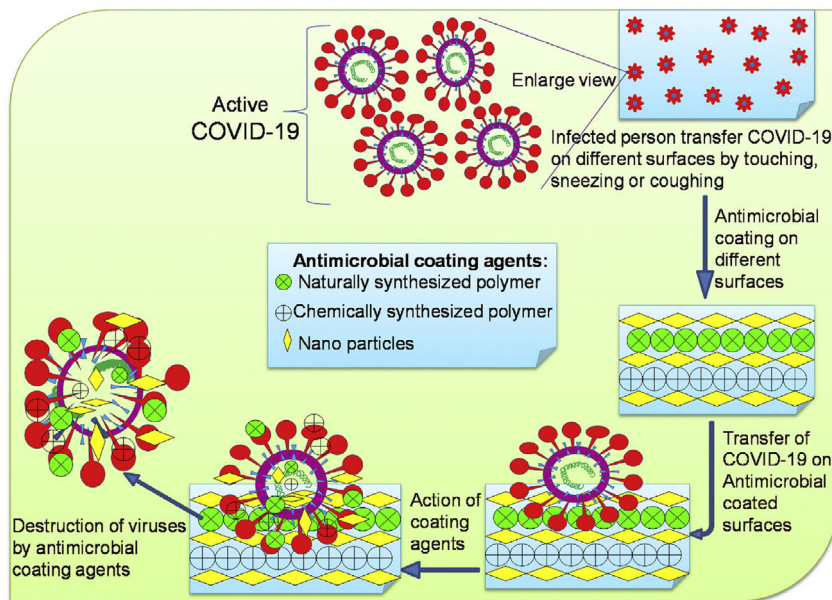


FIGURE 5 The proposed mechanism to prevent the spread of the COVID-19 on surfaces through nanocoatings (reproduced from ref. <sup>309</sup> with permission from Elsevier)

## 7.2 | Nanotechnology applications in the detection of coronaviruses

Nanomaterials, due to their small size and large surface area, can be effective in sensitivity increase and also quick and easy detection of coronaviruses.<sup>318</sup>

### 7.2.1 | Nanobiosensors

The advantages of combining nanotechnology and biosensors include increased sensitivity and selectivity, improved detection limit, accelerated response, and reduced dimensions of devices, portability, and POC detection.<sup>319–321</sup> Nanomaterials are appropriate for biosen-

sors due to their unique physical and chemical properties and catalytic properties, such as improved electron transfer, biomolecule labeling, and biomolecule adsorption capability.<sup>320</sup> Table 7 presents some nanobiosensors for the detection of human coronaviruses, such as MERS, SARS, SARS-CoV2, and also infectious bronchitis virus (IBV) and influenza.

### 7.2.2 | Using nanomaterials for signal enhancement

Metal nanomaterials, in many cases, are used to amplify the output signal of biosensors and increase sensitiv-

**TABLE 6** Common antiviral and antibacterial nanomaterials along with their mechanisms

| Nanomaterials   | Proposed mechanisms   | References  |
|---|---|-------------|
| Ag nanoparticles (NPs)  | <ul style="list-style-type: none"> <li>The silver ion release</li> <li>Alteration of microbial membrane permeability</li> <li>Interaction of silver nanoparticles with intracellular proteins, especially membrane proteins containing sulfur and microbial DNA</li> <li>Disruption of cell division leading to cell death</li> </ul>   | 313–315     |
| AuNPs   | <ul style="list-style-type: none"> <li>Contact of AuNPs with viruses has no significant effect on disabling viruses</li> <li>The proposed mechanism is the production of heat by light radiation to gold nanoparticles at a specific wavelength, which is called plasmonic photothermal property, resulting in a change in the virus membrane and a decrease in the binding power of the virus to the surfaces</li> </ul> | 313,314     |
| CuNPs   | <ul style="list-style-type: none"> <li>The Cu ions release reactive oxygen species (ROS) produced from Cu reacting with exogenous hydrogen or molecular oxygen through Fenton-like or Haber Weiss reactions</li> <li>Damage to envelope proteins and lipids</li> </ul>  | 313,314,316 |
| ZnONPs  | <ul style="list-style-type: none"> <li>Intercellular accumulation of nanoparticles</li> <li>Generation of ROS, which accumulates in cell membranes and causes damage to the cell wall and increases membrane permeability</li> <li>Release of Zn ions into the cell and disruption of RNA replication</li> </ul>  | 313,314,317 |
| TiO <sub>2</sub> NPs  | <ul style="list-style-type: none"> <li>Production of ROS by UV light due to the photocatalytic properties of TiO<sub>2</sub>NPs</li> <li>Antiviral activity in the absence of light due to cell uptake by NPs and destruction of their membrane</li> </ul>  | 313,314     |
| Carbon nanostructures (fullerene, graphene, carbon nanotube [CNT], graphene, and diamond-like carbon) | <ul style="list-style-type: none"> <li>Carbon nanostructures damage bacterial cell walls and cell membranes</li> <li>The production of ROS by light radiation due to photochemical activity disrupts cell membranes and damages RNA. It affects the metabolic pathways of energy to inactivate microorganisms in compounds, such as fullerene</li> </ul>  | 313,314     |

ity for their unique properties. Due to their ability to accelerate electron transfer<sup>318</sup> and high conductivity, stability, biocompatibility, and electrical properties related to size, metal nanoparticles can be deposited as a layer on the electrode used in electrochemical biosensors, and their surface can be functionalized with bioreceptors.<sup>323,329</sup>

It is noteworthy that silver and gold nanostructures are applied in optical and colorimetric biosensors as bioreceptors due to their LSPR and PPT properties; otherwise, they are attached to biomolecules to improve the color signal.<sup>322,338</sup>

In this regard, a dual-functional plasmonic biosensor was designed to detect SARS-CoV-2, which operates based on combining the PPT effect and LSPR with gold functionalized nanoislands and complementary DNA receptors. In this project, heat generation due to AuNIs irradiation in plasmonic resonance frequency provides in-situ hybridization of RdRp-CoV and cDNA. According to this study, high

sensitivity, fast response, and improved accuracy are the salient features of this biosensor.<sup>322</sup>

### 7.2.3 | Implementing nanomaterials in the form of field-effect transistor

Semiconductor nanomaterials, due to electronic properties, are sensitive to the binding of biomolecules on the surface in the form of FET. Carbon nanotubes,<sup>333</sup> silicon nanowires,<sup>342</sup> graphene,<sup>324</sup> and transition metal dichalcogenide<sup>343</sup> are typical semiconductor nanostructures for the fabrication of these types of nanosensors that can detect viruses with high sensitivity without the need for viral RNA amplification.<sup>344</sup>

Applying CNT-functionalized fibronectin based on protein for a conductance biosensor construction to detect the SARS virus is one of the applications of these nanomaterials.<sup>331</sup> Also, antibody-coated graphene sheet

TABLE 7 Types of nanobiosensors for the detection of human coronaviruses

| Target   | Type of biosensor | Nanomaterials                                     | Role of nanomaterials  | Methods  | Bioreceptor                 | LOD  | Response time  | POC (Y/N) | References |
|----------|-------------------|---|--|--|-----------------------------|--|----------------|-----------|------------|
| COVID-19 | Optical           | Gold nanoisland with two dimensions               | Functionalizing AuNI with complementary DNA receptors for plasmomic photothermal (PPT) enhancement | Simultaneous use of two techniques: localized surface plasmon resonance (LSPR) and PPT | Nucleic acid                | Lower than 0.22 PM                             | –              | N         | 322        |
|          | Electrochemical   | Gold nanoparticles                                | Drop cast onto the fluorine doped tin oxide (FTO) electrode as signal amplifier                    | Potentiometric FTO-based immunosensor  | Antibody                    | 120 FM   | 20 s           | N         | 323        |
|          | Electronic        | Graphene sheets                                   | Coating graphene sheets of field-effect transistor (FET) with antibody                             | FET/voltammetric biosensor   | Antibody                    | $2.42 \times 10^2$ copies/m (for medical test) | –              | Y         | 324        |
|          | Colorimetric      | Dye-coated polymer nanoparticles                  | Dye label for streptavidin   | RT-LAMP coupled with nanoparticles-based lateral flow biosensor                        | Nucleic acid                | 12 copies of each reaction                     | 1 h            | Y         | 325        |
|          | Optical           | Lanthanide-doped polystyrene nanoparticles (LNPs) | Self-assembled LNPs for labeling antibody  | Fluorescent LFA immunoassay  | Nucleocapsid phosphoprotein | –  | 10 min         | N         | 326        |
|          | Colorimetric      | Gold nanoparticles                                | AuNPs for labeling antigens  | Lateral flow immunoassay for simultaneously detection of IgM and IgG antibodies        | COVID-19 antigen            | –  | 15 min         | Y         | 327        |
| MERS     | Optical           | Nanopillar arrays (NPA) comprise gold nanoislands | NPA enhances light absorption, and functionalized AuNI provides heating for PCR                    | PPT heating for ultrafast PCR on-chip  | Nucleic acid                | $0.1 \text{ ng}\cdot\mu\text{l}^{-1}$          | 3 min and 30 s | Y         | 328        |
|          | Electrochemical   | Gold nanoparticles                                | Depositing AuNPs on the electrode surface for signal enhancement                                   | Voltammetric immunoassay chip  | Spike protein S1            | $1.0 \text{ pg}\cdot\text{ml}^{-1}$            | 20 min         | Y         | 329        |

(Continues)

TABLE 7 (Continued)

| Target    | Type of biosensor | Nanomaterials                           | Role of nanomaterials  | Methods   | Bioreceptor                              | LOD                                   | Response time | POC (Y/N) | References |
|-----------|-------------------|---|--|---|--|---------------------------------------|---------------|-----------|------------|
|           | Colorimetric      | Gold nanoparticles                      | Functionalized AuNPs with dsDNA self-assembly  | LSPR  | Nucleic acid                             | 1 pmol/ $\mu$ l                       | 10 min        | Y         | 330        |
|           | Colorimetric      | Gold nanoparticles                      | AuNP-bound polyHRP for signal enhancement  | Simultaneous use of ELISA and LFA on 2-dimensional paper network  | HRP-conjugated antibody label            | $5 \times 10^{11}$ NA copies/ml       | 60 min        | Y         | 331        |
|           | Colorimetric      | AgNPs                                   | Using aggregation property of NPs for color changing                                     | Paper-based colorimetric assay-based nanoparticles aggregation  | Pyrrolidinyl peptide nucleic acid        | 1.53 nM                               | –             | Y         | 332        |
| SARS      | Electronic        | Carbon nanotube                         | Carbon nanotube functionalization with fibronectin-based protein                         | FET/conductance biosensor   | Nucleic acid                             | 5 nM                                  | –             | Y         | 333        |
|           | Electronic        | In <sub>2</sub> O <sub>3</sub> nanowire | In <sub>2</sub> O <sub>3</sub> nanowire functionalization with fibronectin-based protein | FET/voltammetric biosensor  | Antibody mimic proteins (AMPs)           | 0.6 nM of N protein in 44 $\mu$ M BSA | 10–15 min     | N         | 334        |
| Influenza | Optical           | CdSe/ZnS quantum dots (QDs)             | QDs labeled antibody   | Use of QDs as fluorescent labels in LFIAS   | Antibody                                 | 0.01 ng/ml                            | 15 min        | Y         | 335        |
|           | Electrochemical   | CdS QDs                                 | Influenza virus labeled with QDs   | Pulse voltammetry and pulse anodic stripping voltammetry  | Glycon-modified MPs in streptavidin chip | –                                     | –             | N         | 336        |
|           | Optical           | QDs                                     | QD labels excited with UV LEDs   | LFA/ratiometric mobile phone fluorescence imaging   | Antibody                                 | 2 fmol                                | –             | Y         | 337        |
|           | Optical           | Ag@SiO <sub>2</sub> nanoparticles       | Functionalized NPs as a metal-enhanced fluorescence sensing platform                     | Surface plasmon resonance enhancement, which can be transformed into more efficient fluorescence emission | Guanine-rich anti-Rha aptamer            | 2–3.5 ng/ml                           | 30 min        | Y         | 338        |

(Continues)

TABLE 7 (Continued)

| Target | Type of biosensor | Nanomaterials  | Role of nanomaterials   | Methods   | Bioreceptor                   | LOD                              | Response time | POC (Y/N) | References     |
|--------|-------------------|--|---|---|-------------------------------|----------------------------------|---------------|-----------|----------------|
| IBV    | Electrochemical   | Iron magnetic nanoparticles/gold nanoparticles   | MNP-influenza virus-AuNPs sandwich  | Chronoamperometric biosensor  | Anti-M2 antibody and Fetuin-A | Less than 16 HAU                 | 160 s         | Y         | <sup>339</sup> |
| IBV    | Optical           | Chiral zirconium QDs and magnetoplasmonic nanoparticles (Fe <sub>3</sub> O <sub>4</sub> @Au) | Formation of nanostructured magnetoplasmonic fluorescent with the addition of target      | Using the fluorescence properties of immunoconjugated QD-MPNPs nanohybrids      | Antibody                      | 79.15 EID/50 ml                  | –             | N         | <sup>340</sup> |
| IBV    | Optical           | Molybdenum disulfide 2-D nanosheet   | Fluorescence-quenching ability of MoS <sub>2</sub> when applied to a dye-labeled antibody | Fluorescent immunosensor performed on cotton thread-based microfluidic platform | Antibody                      | 4.6×10 <sup>2</sup> EID50 per ml | 10 min        | Y         | <sup>341</sup> |

has led to the development of a voltammetric biosensor for SARS-Covid 2 detection.<sup>324</sup>

#### 7.2.4 | Using nanomaterials for labeling biomolecule

Nanoparticles that attach to biomolecules as labels in optical nanobiosensors have properties such as fluorescence.<sup>341</sup> In optical biosensors, nanoparticles with fluorescence properties, especially QDs, are used. QDs and AuNPs have also been used as labels in electrochemical biosensors due to their electrical properties.<sup>336,339</sup> In one study, an IBV detection method using fluorescence and magnetoplasmonic nanocrystals was proposed. In this method, after preparing ZrQDs and paramagnetic nanoparticles and binding IBV antibodies, these nanoparticles come together and form a magnetoplasmonic fluorescent nanohybrids structure in the presence of the target virus. They could be separated from the solution with an external magnet and by measuring their photoluminescence intensity, and the targeted analytic concentration can also be calculated.<sup>340</sup>

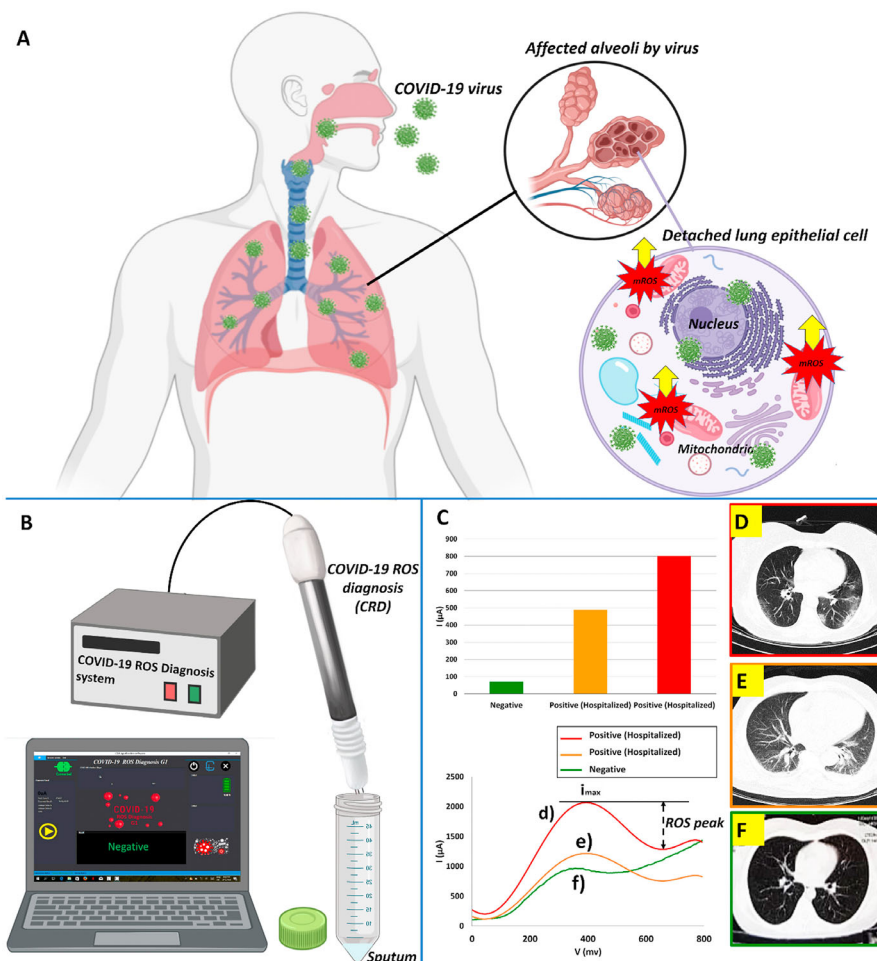
Another biosensor for IBV detection was made of MoS<sub>2</sub> that is a 2-D nanosheet with a high ability to turn off fluorescence when attached to a dye-labeled antibody. This immune sensor utilized fluorescence resonance energy transfer between MoS<sub>2</sub> and the antibody-bound dye label during antibody–antigen interaction.<sup>341</sup>

#### 7.2.5 | Using aggregation property of nanoparticles in colorimetric nanobiosensor

A label-free colorimetric method for detecting MERS-CoV has been developed using the optical properties of gold nanoparticles that lead to color change in the presence and absence of the target molecule. In a recent study surface of the gold, nanoparticles were coated with two thiolated single-stranded DNAs with strong Au–S interaction. Hybridization of target DNA with ssDNA on gold nanoparticles' surface leads to AuNPs aggregation and changes the solution's color through an LSPR shift.<sup>330</sup>

Other applications of nanotechnology include nanomaterials used as a tracer in biochemical sensors. One of the biosensors for detection of CoV-19 used a multiwall carbon nanotube (MWCNT) to cover the electrode heads in the biosensor. The biosensor was based on electrochemical measurements of ROS released in a virus-infected lung. MWCNT is known as an electrochemical superoxidant selective tracer, such as H<sub>2</sub>O<sub>2</sub>/ROS. The amount of electrical change is characterized due to the ROS reaction on the surface of the electrode and is transferred to the





**FIGURE 6** Schematic illustration of: (A) COVID-19 side effect in lung host cells (the virus amplifies viral replications by inducing overproduction of mitochondrial ROS); (B) the COVID-19 ROS diagnosis system (consists of three needle electrodes coated by functionalized MWCNTs); (C) electrochemical reaction and cyclic voltammetry cathodic peaks from two different patients; (D and E) the CT-scan of the patient showed more distinctive hazy patches; (F) a normal candidate lung CT-scan (reproduced from ref. <sup>345</sup> with permission from Elsevier)

working electrode by the counter electrode, and this system measures the released ROS by cyclic voltammetry procedure (Figure 6). The detection time is less than 30 s in this biosensor.<sup>345</sup>

### 7.2.6 | Extraction of RNA by magnetic nanoparticles

RT-PCR test requires the extraction of high-purity viral RNA from complex samples.<sup>346–348</sup> This method is entirely dependent on the amount of target so that if the extraction efficiency is low, the received signal as a response will be weak and may lead to false-negative results.<sup>349</sup> The silica-based spin column is a standard method for extracting RNA from nasopharyngeal cells.<sup>346,349</sup> This method protocol has several steps, such as sample prelysis, centrifugation, washing, and consumption of toxic organic solvents, which is time-consuming and requires plenty of opera-

tors in the laboratory. Due to low extraction efficiency and operator endangerment, it is essential to use a fast and automatic technique to extract viral RNA.<sup>346,350,351</sup> Surface functionalized magnetic nanoparticles (MNPs) can extract viral RNA as an alternative method.<sup>352</sup> This method has fewer steps, does not require any centrifuge step, fast and straightforward, and can also be operated manually and automatically.

Additionally, it has high extraction efficiency and reduces false-negative results. In this method, the surface of MNPs is functionalized by groups with strong interaction with nucleic acids and RNA molecules are efficiently and rapidly adsorbed from the lysis solution on the surface of MNPs. The extracted RNA can enter the detection process by RT-PCR without MNPs wash or after washing with buffer and separated nanoparticle.<sup>346,350,353</sup>

In one study, by using the poly amino ester-functionalized  $\text{Fe}_3\text{O}_4$  NPs, viral RNA molecules were adsorbed on the surface of NPs via carboxylic groups and

pcMNPs–RNA complex was used directly in RT-PCR for the detection of SARS-CoV-2.<sup>346</sup>

### 7.3 | Nanotechnology-based treatments

Today, various nanotechnology-based products have been produced, developed, and made available to the public in order to fight COVID-19.

#### 7.3.1 | Vaccine development

The development of viral or peptide vaccines against various diseases, such as infectious diseases, has been a medical advancement.<sup>354–356</sup> Nanotechnology has the potential for the development of COVID-19 drug delivery due to enormous advantages included:

1. Small size and morphology of the nanoparticles that can deliver the drug to physiologically sites and reduce immune response by reticular endothelial cells.<sup>357</sup>
2. The big surface-to-volume ratio of nanoparticles that make an increase of drug loading.<sup>358</sup>
3. The ability to cross negatively charge membranes because of surface charge modification of nanoparticles.<sup>359,360</sup>

According to many studies, both humoral and cell-mediated immunity play a beneficial role in SARS-CoV infection.<sup>361,362</sup> Both adaptive systems (T cells and B cells) and innate immune systems (macrophages, monocytes, and neutrophils) at the cellular level can be targeted by nanoparticles. Modulating APCs using nanoparticles could be very significant, especially for COVID-19 vaccine strategies.<sup>363,364</sup> The capability of nanoparticles to deliver antigen to DCs by enhancing antigen presentation and several other mechanisms can boost T cell immunity.<sup>365</sup> Various nanoparticle-based mechanisms for altering the induction of the immune response have been described in many studies. Nanoparticles have shown many benefits for improving the efficacy and safety of the vaccine approach, including their ability to deliver molecular adjuvants. Also, in some cases, the nanomaterials themselves have inherent adjuvant property for the loaded antigens.<sup>366</sup> Table 8 summarizes the applications of different nanoparticles against COVID-19, SARS, and influenza.

#### 7.3.2 | Therapeutic development

Nanotechnology offers many solutions to prevent COVID-19. In this section, we will review some solutions in this field.

#### *Use of carbon quantum dots for COVID-19 treatment*

In one study, carbon quantum dots (CQDs) were first synthesized and post modified with boronic acid ligands. The second generation of anti-HCoV nanomaterials contains CQDs derived from 4-aminophenylboronic acid without any further modification, which has an EC<sub>50</sub> lower than  $5.2 \pm 0.7 \mu\text{g ml}^{-1}$ . The main mechanism of action of these CQDs is inhibition of HCoV-229 entry, which occurs due to the interaction of its functional groups with HCoV-229 entry receptors, so that an equally large inhibition activity is observed in the viral replication phase.<sup>376</sup>

#### *Mesenchymal stem cells and management of COVID-19 pneumonia (LIFNano)*

Leukemia inhibitory factor (LIF) is essential to resist the cytokine storm in the lungs in the period of viral pneumonia.<sup>377,378</sup> Even though MSCs produce LIF as a remedy, this fails because of cell-based nature while bringing a prohibitive cost burden. Based on nanotechnology strategies, synthetic stem cells “LIFNano” can be used with a thousand times boost in effectiveness than soluble LIF.<sup>379</sup> In EAE, a preclinical version of multiple sclerosis, LIFNano-based therapy navigated paralysis in 1 week, a timeline in accordance with that revealed for advantageous impacts in COVID-19 pneumonia using MSC treatment. Earlier experiments using neural stem cells (NSCs) to cure experimental autoimmune encephalomyelitis (EAE) indicated advantages that were only related to NSC-derived LIF. While a developing replacement to cell-based therapy, like LIFNano, provide the requirement for a large quantity and off-the-shelf medicinal agents able to regenerate injured tissues and restrain cytokine storm in pneumonia. Worldwide delivery is straightforward utilizing low-volume vials optional delivery routes, including inhalation or intravenous or both.<sup>380</sup>

## 8 | IN-SILICO STUDIES AND COMPUTATIONAL BIOLOGY APPROACHES IN COVID-19

### 8.1 | Background

The silico study allows the researcher to perform an utterly complete classification of various parameters, thereby providing further suggestions or predictions of workable results.<sup>381</sup> Thus, bioinformatics resources have become a powerful tool for optimizing and analyzing the interaction between target proteins and antiviral candidates, searching for new antiviral compounds by reducing the time and costs associated with laboratory evaluation.<sup>382</sup>

**TABLE 8** Applications of nanoparticle-based vaccine-like polymeric NPs, peptide-based NPs, inorganic NPs, and so on against COVID-19, SARS, and influenza

| Target    | Nanomaterials                                       | Antigen  | Mechanism   | Adjuvant                | References |
|-----------|---|--|---|-------------------------|------------|
| COVID-19  | Au NPs  | Swine transmissible gastroenteritis                  | Rise of the peritoneal macrophages respiratory action and plasma IFN- $\gamma$ level  | Au NPs                  | 367        |
|           | Au NPs  | SARS-CoV   | Induction of IgG response   | Au NPs                  | 368        |
|           | Ferritin-based NPs                                  | MERS-CoV (RBD antigen)                               | Initiation of CD4 <sup>+</sup> T cells and IFN- $\alpha$ TNF- $\gamma$ reactions  | –                       | 369        |
|           | Spike protein NPs                                   | MERS-CoV   | Initiation of better titers of neutralizing antibody and Th2 immune response with no initiation of Th1 immune reaction  | Aluminum                | 370        |
|           | Hollow polymeric NPs                                | MERS-CoV (RBD antigen)                               | Initiation of elevated amounts of humoral reactions and IgG2a antibodies with no initiation of lung eosinophilic immunopathology  | –                       | 371        |
| SARS      | Polyprotein 1a (pp1a) of the human SARS coronavirus | Proteins 10 and 11 (hereafter NSP10)                 | Used as a novel self-assembling system for NPs  | Adavax (Squalene based) | 372        |
| Influenza | Poly(lactic-coglycolic acid)                        | Inactivated SwIV H1N2                                | (PLGA-KAg) capsule vaccine in cells in vitro  | –                       | 373        |
|           | Au NPs  | Antigen extracellular domain of the M2 protein (M2e) | Use gold nanoparticle and its conjugated CpG oligodeoxynucleotide 1826 to document rises in the breathing action of splenic lymphocytes in the respirational action of peritoneal macrophages and in the creation of proinflammatory cytokines (IL-6 and IFN- $\gamma$ ). | CpG                     | 374        |
|           | Virus-like particle                                 | Hemagglutinin  | Extremely preserved multiple ectodomains of matrix protein 2 (M2e5x VLP) of influenza virus stimulate broad cross-protection by M2-specific humoral and cellular immune reactions   | –                       | 375        |

## 8.2 | Computational biology and bioinformatics in fundamental studies of SARS-CoV-2

In-silico findings and computational studies have greatly accelerated the process of fundamental studies of SARS-CoV-2, such as virus genomics and proteomics, structural studies, evolutionary studies, and phylogenetics. These necessary studies provide an introduction to the rapid development of research in the diagnosis, prevention, and treatment of COVID-19, each of which is facilitated by in-silico approaches. In the field of genomics and proteomics

of SARS-CoV-2, various databases and servers provided services, which can be seen in Table S2.

A simple search in the NCBI database with SARS-CoV-2 keyword to date revealed that one genome (Accession number: NC\_045512.2), 25,501 nucleotide sequences, one taxonomy, 12 genes, 135 GEO data sets, 265,862 protein sequences, 457 protein structures, and 3711 case clinical trials have been recorded, which indicates an explosion of information in this case.

Some servers have also been instrumental in the structural modeling of SARS-CoV-2 proteins or their derivatives, such as Phyre 2 for ab initio modeling, PSIPRED

for prediction of secondary structure, and ITASSER and PEPFOLD3 for predicting the third structure (3D) of proteins and peptides and the SWISS-MODEL for the homology modeling of sequences whose structures are unknown.<sup>383–387</sup>

The design of specific primers (by bioinformatics tools) to perform molecular detection tests on nasopharyngeal and oropharyngeal specimens was one of the first in-silico achievements in COVID-19. From the very beginning, qRT-PCR was the basis for this type of diagnosis that the primers based on ORF1ab and N genes were designed.<sup>388</sup> In the diagnosis of the SARS-CoV-2, false-negative responses have a high rate and diagnostic accuracy is about 30–50%, which is due to mutations and the spread of different types of viruses. Accordingly, more detailed studies are being conducted in this field today, in which bioinformatics studies play an essential role.<sup>389,390</sup> For example, to select the best regions for primers design, conserve areas in cDNAs derived from different SARS-CoV-2 variant genomes can be used, which are obtained after alignment with bioinformatics methods, such as FASTA, Basic Local Alignment Search Tool, and multiple sequence alignments, such as Clustal Omega and T-Coffee.<sup>391–395</sup> However, this strategy assumes that DNA sequences have general characteristics and require prior information and, usually, several tests for accurate diagnosis.<sup>396</sup>

Deep learning (DL) methods based on the artificial neural networks (ANNs) machine learning (ML) algorithm can have many applications in bioinformatics studies, drug design, and biomedical analysis.<sup>397–399</sup> DL methods are essential strategies for classifying viral DNA and cDNA sequences without the need for prior information.<sup>400,401</sup> In April 2020, a study was published in the Bulletin of the World Health Organization that used a combination of DL techniques, viromics, and primer design with bioinformatics tools to design specific primers for accurate SARS-CoV-2 detection. Their results showed that designed primers are very specific and significantly increase the accuracy of the diagnosis of COVID-19 compared to previous studies.<sup>391</sup> In addition to genome and proteome analysis by ML algorithms, a method called nCOVnet uses an ANN-based DL strategy to screen and rapidly analyze X-ray images of the lungs of people suspected of having COVID-19 for definitive diagnosis.<sup>402</sup>

### 8.3 | Immunoinformatics technics and in-silico studies in vaccine design for COVID-19

In the prevention and design of vaccines or multiepitope construct with diagnostic value, immunoinformatics studies are critical in identifying the best immune-dominant

epitopes of different SARS-CoV-2 proteins.<sup>403–405</sup> In the early 1990s, a genome-based vaccine design or reverse vaccinology approach was introduced. Today, bioinformatics methods have facilitated the development of this approach.<sup>406</sup> In many in-silico studies to COVID-19 vaccine design, epitopes associated with T helper and Cytotoxic T lymphocytes (CTL) epitopes were placed next to B cell epitopes by specific linkers so that the final construct could stimulate both humoral and cell-mediated immunity and produce a better immune response.<sup>405,406</sup> Many servers and software have been useful in this area.<sup>403–405</sup>

In this regard, in addition to actual databases, such as NCBI, PDB, ExPasy, and PDB sum, other servers provide immune-informatics services to design multiepitope constructs. For example, Vaxijenserver<sup>407</sup> for predicting protein antigenicity, Immune Epitope Database (IEDB)<sup>403–405,408</sup> for mapping, forecasting, and evaluating epitopes, and NetCTL1.2 for designing CTL cell epitopes are useful.<sup>404,409</sup> Awareness of Human leukocyte antigen (HLA) types involved in the immune response to COVID-19 is essential for vaccine design. TepiTool from IEDB server can enable this.<sup>410</sup> Epitool kit is a suitable server for designing Treg cell epitopes.<sup>404,411</sup> ABCpred and BepiPred linear epitope prediction tools can create linear B cell epitopes,<sup>412–414</sup> while Ellipro is used to design conformational (discontinuous) B cell epitopes.<sup>415</sup> AlgPred and Allertop servers are used to predict the allergenicity of designed sequences.<sup>416,417</sup> Usually, the final construct's antigenicity is rechecked with Vaxijen v2.0 or ANTIGEN-pro tools.<sup>407,418</sup> The SOLPro server predicts the solubility of the protein construct.<sup>419</sup> C-ImmSim online server is used to simulate the immune response against the final design.<sup>420</sup> Phyre 2 and Galaxy refine server can predict the structure, modify, and evaluate the quality of the spatial structure of the final multiepitope construct.<sup>421,422</sup> Prot-Parm can predict the physicochemical properties of the designed construct. The most important of these properties are isoelectric pH (pI), molecular weight (Mw), amino acid content, hydrophobicity, instability index, and Grand average of hydropathicity index (GRAVY).<sup>423</sup> RAMPAGE and SWISS-MODEL can be used to predict the placement of protein backbone residues according to their torsion (dihedral) angles in the permitted or unauthorized areas of the Ramachandran plot.<sup>404,424</sup> The ProSA-web server can also be used for the analysis and final validation of the structure of a designed protein construct.<sup>425</sup>

To evaluate the interaction affinity and binding stability of vaccine candidate construct with some important immune-receptors, such as Toll-like receptors (TLRs) and Human leukocyte antigens (HLAs), molecular docking studies and molecular dynamics (MD) simulations are very important and based on their results can be selected as the best vaccine candidates.<sup>404,426,427</sup> HADDOCK and ClusPro

are useful methods for performing molecular docking between two proteins or a peptide with one protein.<sup>428–430</sup> GROMACS and Yet Another Scientific Artificial Reality Application (YASARA) are also two servers suitable for MD.<sup>431,432</sup> Eventually, at the IEDB, Population Coverage Analysis Tool can be performed to determine what percentage of the population responds appropriately to the epitopes.<sup>404</sup> The population coverage analysis tool calculates the fraction of individuals predicted to respond to a given set of epitopes with known MHC restrictions.<sup>433</sup>

## 8.4 | Chemoinformatics and computational bioinformatics for drug discovery and drug design to combat COVID-19

Computer-aided drug discovery is a promising strategy for developing novel drugs and specific targets to combat diseases, such as COVID-19; this issue requires understanding of the structure of coronavirus and various target proteins.<sup>434</sup> Regarding the urgency of therapeutic measures for the COVID-19 pandemic and because drug discovery is a time-consuming process, the use of available drugs with FDA approval is preferred due to the need for less cost and time.<sup>435</sup> In-silico medicine repurposing represents an accurate way to speed up the screening of existing drugs with FDA approval to find a therapeutic option for COVID-19.<sup>436,437</sup>

For this purpose, databases and servers related to drug and chemical compounds, such as DrugBank (<https://go.drugbank.com/>) and PubChem (<https://pubchem.ncbi.nlm.nih.gov/>), were used as beneficial resources. Also, chemoinformatics and in-silico approaches, such as Monte Carlo-based quantitative structure–activity relationship (QSAR), SARS-CoV-2 Genome Annotation (NCBI server), Homology Modeling (SWISS-MODEL), virtual screening (VS), molecular docking (Large-Scale Docking by supercomputer), MD, prediction of physicochemical properties, toxicity, immunogenicity, pharmacokinetic/pharmacodynamics parameters, and drug-likeness analysis, have been widely used in drug discovery and design for COVID-19.<sup>438,439</sup>

Virtual screening has become a popular method for novel drug discovery and drug repurposing.<sup>393,394</sup> Structure-based and ligand-based drug discovery and design are two critical subgroups of this type of virtual screening.<sup>440,441</sup> Usually, in silico, drug screening for COVID-19 is performed based on structures of viral proteins (spike protein, viral helicase, 3C-like protease [3CL<sup>pro</sup>], and RNA-dependent RNA polymerase order) and human ACE2 receptor.<sup>439</sup> In drug discovery, in addition to HADDOCK and ClusPro mentioned above,

AutoDock Vina is widely used for molecular docking protein targets and small molecules.<sup>442</sup>

The big data obtained from high-throughput screening and the high number of drug candidates have shown that ML algorithms, such as support vector machine, ANN, random forests, discriminant analysis, and so on, have become a fundamental tool for drug designing and discovery.<sup>423,443</sup> ML techniques can be used to model QSAR or quantitative structure–property relationships and develop artificial intelligence programs that accurately predict in silico how chemical modifications might influence biological behavior.<sup>441,442</sup> Many physicochemical properties of drugs, such as toxicity, metabolism, drug–drug interactions, and carcinogenesis, have been effectively modeled by QSAR techniques.<sup>443,444</sup>

Finally, on the importance of in-silico drug discovery and repurposing, it should be noted that due to the lack of specific FDA-approved drugs against SARS-CoV-2, currently available antiviral drugs, such as remdesivir, favipiravir, CQ, and so on, are prescribed to COVID-19 patients.

## 9 | CONCLUSION AND FUTURE PERSPECTIVE

This article reviews the general and biological characteristics of the virus, including genomics, proteomics, receptors, immunopathology, host immune response, and pathogen immune evasion strategies. Then, various molecular methods, including nucleic acid- and protein-based as well as the POC and X-ray-based methodologies examined in the diagnosis of COVID-19, were discussed. In the following, two pathways for manipulation of the mice for SARS-CoV-2 studies were described, including producing hACE2 mice for the development of vaccines and other potential antiviral therapies and knockout of other genes to mimic disease. Then, we framed the symptom effects and clinical properties of COVID-19 on various conditions. Symptoms and illnesses were included in abdominal symptoms, hypertension, diabetes, renal dysfunction, cancer, and so on. In the next section, multiple treatments for this disease were described, including therapeutic and antiviral agents and inhibitors, supporting agents, and other potential therapies. Then, the applications of nanotechnology in the identification, diagnosis, and treatment of coronaviruses and, finally, in-silico studies and biocomputational approaches in COVID-19 were reviewed. According to the extensive studies that are being done around the world during the outbreak of this disease, there is no doubt that effective drugs and vaccines will be made for this disease, although some of them have entered the consumer market. The use of new technologies, such as nanotechnology, will also facilitate the continuation,

diagnosis, and treatment of COVID-19. Nevertheless, we should confess that the unexpected time-to-time mutations and designation of new variants of COVID-19 make it, indeed, a near-to-impossible task to look at the future ahead of treatment and mortality prevention of COVID-19 from an explicit perspective.

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#### CONFLICT OF INTEREST

There is no conflict of interest to declare.

#### AUTHOR CONTRIBUTIONS

H.A.M.A. and R.E.-K. conceived the study, collected the literatures, and drafted the manuscript. Corresponding authors, including V.K., H.M., and M.M., provided their corrective comments and tips. All authors collaborated to write the article. P.Z., M.R.S., and M.M. edited the revised version of the manuscript. All authors approved this manuscript for publication.


#### ETHICS APPROVAL


Not applicable.

#### DATA AVAILABILITY STATEMENT

The data included in this study are available upon request from the corresponding authors.

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## SUPPORTING INFORMATION

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