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Comprehensive Review on Current Interventions, Diagnostics, and Nanotechnology Perspectives against SARS-CoV-2

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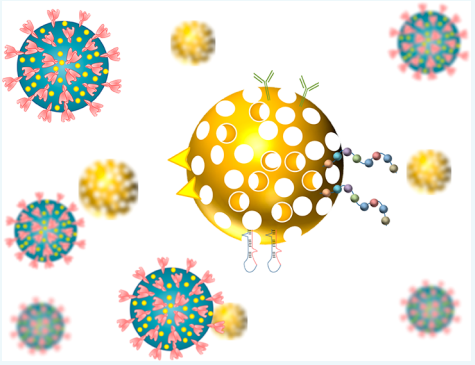
ABSTRACT: The coronavirus disease 2019 (COVID-19) has dramatically challenged the healthcare system of almost all countries. The authorities are struggling to minimize the mortality along with ameliorating the economic downturn. Unfortunately, until now, there has been no promising medicine or vaccine available. Herein, we deliver perspectives of nanotechnology for increasing the specificity and sensitivity of current interventional platforms toward the urgent need of quickly deployable solutions. This review summarizes the recent involvement of nanotechnology from the development of a biosensor to fabrication of a multifunctional nanohybrid system for respiratory and deadly viruses, along with the recent interventions and current understanding about severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).



1. INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is popularly named as novel coronavirus 2019 disease (nCoV-2019).[1] The emergence of coronavirus disease 2019 (COVID-19) is not the first pandemic due to coronaviruses. In the past decade, the outbreak of severe acute respiratory syndrome (SARS, 2002 and 2004) and Middle East respiratory syndrome (MERS, 2012) has shown the capability to cross the interspecies barrier and infect humans. Different from earlier coronavirus outbreaks, COVID-19 has spread to more than 200 countries with millions of cases and thousands of deaths, sparking international health organizations to declare a global health emergency.[2] It appears that the moderate mortality rate, high infection rate, and longer incubation period of SARS-CoV-2 are the perfect combination for prolonging the pandemic. This pandemic can induce a ripple effect and hard challenges to the global health system along with serious financial crises. In many countries, hospitals have been compromised with a shortage of doctors, nurses, and trained personnel due to crowding of COVD-19 patients. Almost all countries are facing the issue of shortage of personal protective equipment (PPE), ventilators, and other critical medical equipment.

Initially, a very low number of cases were reported due to lack of resources and inability to distinguish between common flu and COVID-19. For example, Indonesia has reported only 2 cases.[3] However, there is much tourism from China. Now, several nucleic acid and protein-based diagnostic tests are available for COVID-19. A few repurposed drug candidates are also under clinical trials, but none of them have shown good efficacy without major safety issues.[4,5] Additionally, some of the vaccines have entered phase I, phase II, and even phase III clinical trials.[6] Apparently, the final approval may take more than 12 months; realizing the sense of urgency, interdisciplinary and deployable solutions are highly sought to control this pandemic.

Nanotechnology, an interest of chemistry, physics, biology, and medicine disciplines, emerged as a medically viable approach to enhance the efficacy of the system(s) with minimal efforts and resources. In general, nanotechnology-based interventions increase the specificity, selectivity, sensitivity, and multiplexing characteristics of the diagnostic tests.[7−9] In terms of therapeutic perspectives, nanotechnology can help to develop targeted delivery of therapeutics to avoid the severe systemic side-effects of the regimen.[10] The recent extensive exploration of the drug carriers toward enhancing their biocompatibility, biodegradability, and eco-friendly nature has opened the door of nanotechnology for a wide range of healthcare applications. Overall, nanotechnology advancements can be helpful not only to track COVID-19 but to offer innovative and affordable therapeutic solutions. Apart from therapeutic perspectives, there is a large scope of nano-

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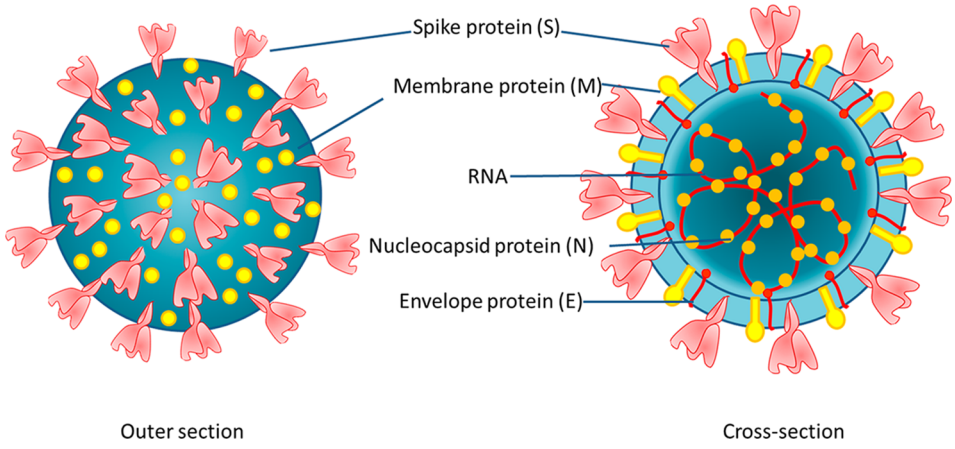

Figure 1. Schematic representing the structure and morphology of SARS-CoV-2.

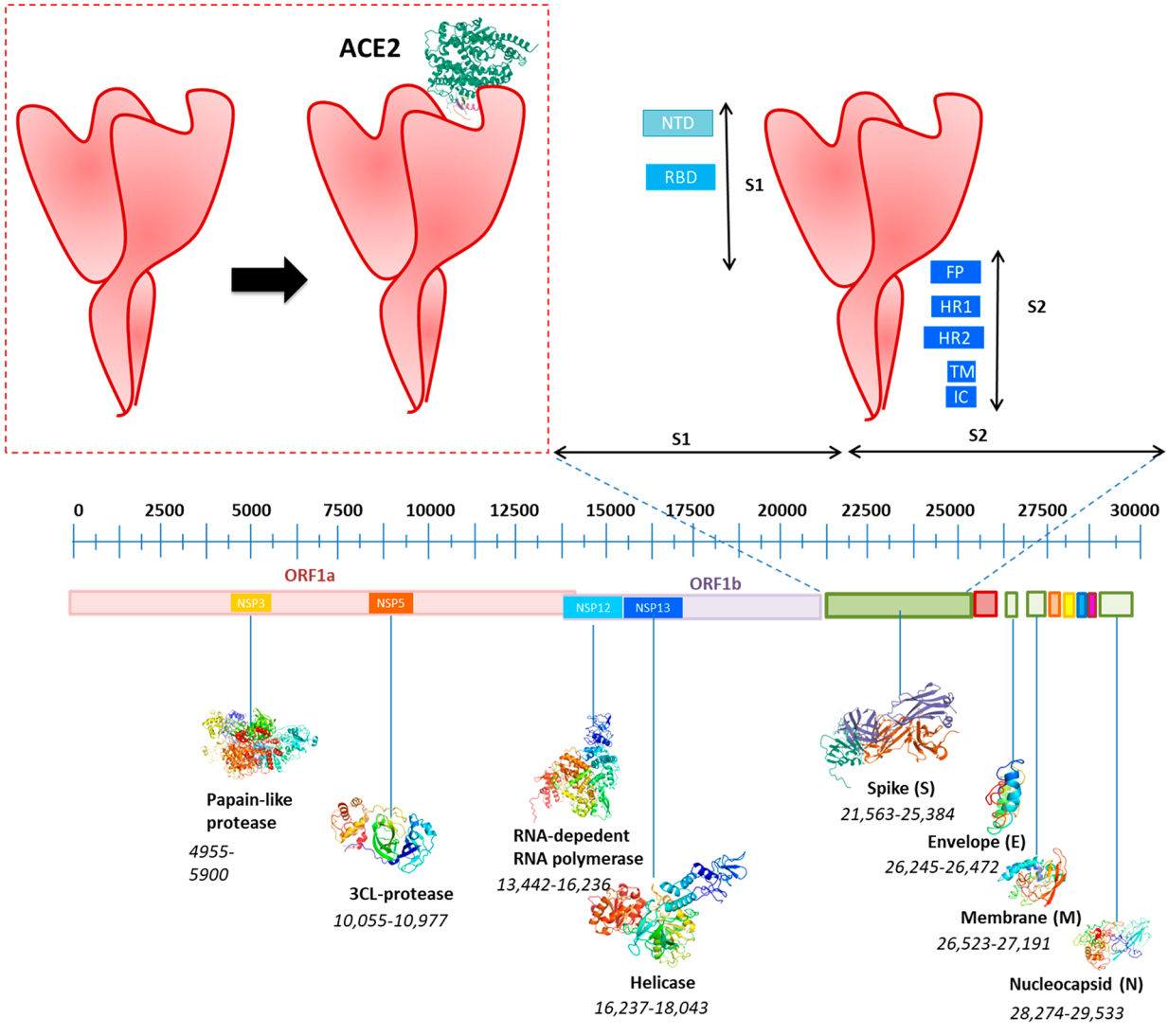

Figure 2. SARS-CoV-2 genome organization, codified proteins, and binding of spike protein to ACE2 receptor. Inset: illustration of ACE2 interaction with the RBD of SARS-CoV-2. Abbreviation: S1, receptor binding subunit, S2. membrane fusion subunit; NTD, N-terminal domain; RBD, receptor binding domain; FP, fusion peptide; HR1, heptad repeat 1; HR2, heptad repeat 2; S1, receptor binding subunit; S2, membrane fusion subunit; TM, transmembrane anchor; IC, intracellular tail; NSP, nonstructural protein.

technology in early and accurate detection of COVID-19. In addition, nanotechnology can be applied for prevention purposes, which includes disinfecting surfaces to nanotextile coating for achieving improved viral inhibition or trapping.

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Keeping the unmet clinical needs of the current pandemic, we aim to document various aspects, such as etiology, pathogenicity, repurposed drugs, biologics, and diagnostic and prevention techniques of COVID-19. Additionally, this review is composed in reference to nanotechnology advancements for helping researchers relate things together and follow up their research accordingly.

2. COVID-19: EPIDEMIOLOGY, ETIOLOGY, AND PATHOGENESIS

COVID-19 is primarily linked to an increase in pneumonia cases in the hospitals of Wuhan, and the sources were traced to the Huanan seafood market.[11] On December 12, 2019, the first COVID-19 patient was declared to suffer from "unexplainable pneumonia", and later, 27 viral pneumonia cases with similar symptoms were officially announced on December 31, 2019. On January 22, 2020, the pathogen has been declared as a novel coronavirus SARS-CoV-2, which originated from wild bats.[12,13] An earlier SARS outbreak occurred during the Spring Festival of China (January 17 to February 23, 2003). Similarly, COVID-19 was also detected and lasted the duration of the festival. A record-high transmission of viral disease and its associated death was primarily due to an increase in the number of travelers (3.11 billion vs 1.82 billion).[1] This became a pandemic in almost all countries due to extensive Chinese traveling around the globe.

According to the World Health Organization (WHO), as of July 2, 2020, the total number of COVID-19 cases registered is 10,514,028 with 512,311 deaths worldwide.[14] The human to human transmission of COVID-19 has been detected as spread of virus from either close contact or droplets.[15,16] The reproductive number of viral transmit infection has been determined to be around 2.20 and 3.58.[17] However, further studies need to be conducted to clearly define the dynamics of transmission. The male to female fatality ratio was found to be 2.4:1, and the median mortality age is 70.3, with IQR (65−81) years.[18] The median time from symptoms to death is 14 days, with an average incubation period of 5.2 days. It has been found to be longer (20 days) in under 70-year-old patients. These studies suggest a higher risk of old age compared to younger. Online sources represent that higher mortality was found in African American and Hispanic populations in the United States (US). The major risk factor for COVID-19 is other chronic diseases, such as diabetes, cardiovascular disease, and hypertension.[19]

SARS-CoV-2 is a single-stranded RNA virus with a length of 29,903 bp and a diameter of ∼50−150 nm.[20] The shape of this novel virus varies from spherical to oval, or pleomorphic morphology (Figure 1). This belongs to the subgenus *Sarbecovirus* and genus *Betacoronavirus*. The culture time varies from 4 to 6 days, as observed in human airway epithelial cells and Vero E6/Huh-7 cell lines.[21]

The organization of the RNA is as follows: 5′-leader-UTR-replicase-S-E-M-N-3′-UTR-poly (A) tail with unknown open reading frames; in which S = spike, E = envelope, M = membrane, and N = nucleocapsid[22] (Figure 2). It has 80.26% sequence identity with query coverage over 98% to the human SARS-CoV genome.[23] Pangolins are considered the intermediate host, as the Guangdong pangolin coronavirus has very high sequence similarity in the receptor-binding domain to SARS-CoV-2. The SARS-CoV-2 enters the host cells via angiotensin-converting enzyme 2 (ACE2) receptor[24] (Figure 2). This receptor is highly expressed in the lungs, upper esophageal epithelial cells, enterocytes of the ileum and colon, and kidney tubules. Thus, the respiratory and digestive routes are the potential entry door for infection.[25,26]

A study revealed that spike proteins of SARS-CoV-2 bind to the ACE2 receptors of the airway passage cells, which cause the entry into the cells.[27] ACE2 is a homologue of ACE, an enzyme known for controlling hypertension. It is a transmembrane metallocarboxypeptidase (primary substrate—angiotensin II) and key player in the renin−angiotensin system. Although the binding of SARS-CoV-2 to the ACE2 is not as strong as SARS-CoV, it is above the threshold and sufficient for infection.[28] Recent findings also suggest that some of the folds may bind tighter in comparison to SARS-CoV.[29] ACE2 facilitated entry of SARS-CoV-2 was examined systematically in the HeLa cells and Vero-E6 cells.[16,25,30] Further, it was noticed that the Gln493 residue of the SARS-CoV-2 receptor binding motif helps in maximizing the interaction with the ACE2.[31]

The spike (S) protein is a homotrimer glycoprotein and composed of two subunits responsible for binding to host cell receptor, and fusion of virus and host cell membrane.[22,31,32] At the boundary of the cell membrane, the S protein is cleaved into the S1 and S2 subunits without breaking apart. The S1 subunit binds to the receptor while maintaining the prefusion conformation of the S2 subunit (containing the fusion machinery) for fusion with the host cell membrane. S protein priming is necessary for the fusion of SARS-CoV, i.e., breakage of S protein by cellular protease at the S_2' sites. It leads to the activation of protein by rendering irreversible changes in the confirmation. The extensive decoration of S protein with N-linked glycans helps in the proper folding and thus access to the host proteases (Figure 2). For priming, Transmembrane Serine Protease 2 (TMPRSS2) is responsible.[30] Consequently, the Calu-2 human lung cell lines treated with the camostat mesylate (a serine protease inhibitor) efficiently blocked the partial infection of SARS-CoV-2.

The alveolar type I and II epithelial cells express the ACE2 receptor.[33] However, RNA profiling studies show that viral receptor ACE2 is concentrated in alveolar type II epithelial cells and express 20 other genes helpful in virus replication and transmission. It is important to note that ACE2 is expressed in only 0.64% of all human lung cells. The expression of ACE2 is correlated with the differentiation state of epithelial cells.[34] The fully differentiated cells (apical surfaces expressing more ACE2) are prone to viral infection, while poorly differentiated cells (express less ACE2) remain poorly infected. SARS-CoV-2 infection relates to the state of the cells, ACE2 expression, as well as ACE2 localization. The percentage of ACE2 positive cells is higher in Asians (2.5%) compared to Africans and Caucasians (0.47%).[35] Also, ACE2 expression is higher in males than females.[36]

The lack of correlation between the mRNA expression and enzyme activity of ACE2 has been established using the drugs lisinopril and losartan.[37] Lisinopril treatment was found to increase the ACE2 mRNA level but not activity, while losartan treatment increased both the activity and the expression level of ACE2. Moreover, combination of losartan and lisinopril treatment has shown no increase in activity and offset in the level of ACE2 mRNA. This study suggests the involvement of angiotensin via ACE inhibitor or angiotensin II receptor antagonist (ARB) to regulate the ACE2 expression and activity. The correlation between hypertension drugs, ACE2 expression, and activity needs to be properly established for controlling the COVID-19.

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Table 1. Currently Available Diagnostic Tests for COVID-19

product	company	LOD	time	sensitivity% (LOD dilution)	specificity%
		Nucleic Acid Amplification Tests			
Quest SARS-CoV-2 rRT-PCR	Quest	136 copies/mL	96−120 h	95% (1×)	100
NY SARS-CoV-2 Real-time RT-PCR	Wadsworth Center, NY state	25 copies/reaction	24−72 h	100 (2×)	100
2019-nCoV Real-Time RT-PCR Dx Panel	CDC	1000 copies/mL	24−72 h	100 (1×)	100
AvellinoCoV2	Avellino laboratories	55 copies/μL	24−48 h	100 (1×)	100
COVID-19 RT-PCR test	LabCorp	6.25 copies/μL	24 h	95 (1×)	100
Cobas SARS-CoV-2 Test Roche	Roche	17−58 copies/mL	24 h	100 (1.5×)	100
COV-19 IDx Assay	Ipsum	8500 copies/mL	24 h	100 (1×)	100
RealTime SARS-CoV-2	Abbott	100 copies/mL	4−6 h	100% (1−2×)	100
New Coronavirus RT-PCR	PerkinElmer	8.3 copies/mL	4−6 h	100% (1.5×)	100
GeneFinder COVID-19 RealAmp Kit	OsangHealthcare	0.5 copies/μL	4−6 h	100 (1×)	100
Lyra SARS-CoV-2 Assay	Quidel	800 copies/mL	4−6 h	100 (1×)	100
NxTAG CoV Extended Panel Assay	Luminex Molecular Diagnostics	5000 copies/mL	4 h	95% (2×)	100
TaqPath COVID-19 Combo Kit	ThermoFisher	10 copies/reaction	4 h	100 (1×)	100
Allplex 2019-nCov Assay	Seegene	1250 copies/mL	4 h	95 (1×)	100
Real-Time Fluorescent RT-PCR kit	BGI	100 copies/mL	3 h	100% (1×)	100
Panther Fusion SARS-CoV-2 Assay	Hologic	0.01 TCID50/mL	3 h	100 (1−5×)	100
BD SARS-CoV-2 Reagents	Becton Dickonson	40 copies/mL	2−3 h	100% (3−5×)	100
ePlex SARS-CoV-2 test	GenMark Diagnostics	10 copies/mL	2 h	94.4 (1×)	100
ARIES SARS-CoV-2 Assay	Luminex Molecular Diagnostics	1000 copies/mL	2 h	100 (1×)	100
COVID-19 genesis Real-Time PCR assay	Primerdesign	330 copies/mL	2 h	100% (3−5×)	100
DiaPlexQ 2009-nCoV Detection kit	SolGent	200 copies/mL	2 h	100 (1×)	100
QuantiVirus SARS-CoV-2 Test Kit	DiaCarta	100 copies/mL	2 h	95 (1×)	100
Logix Smart Coronavirus COVID-19 Test	Co-Diagnostics	4290 copies/mL	1−2 h	100 (1×)	100
Simplexa COVID-19 Direct	Diasorin Molecular	500 copies/mL	1 h	100 (1×)	100
QIAstat-Dx Respiratory SARC-Cov-2 panel	Qiagen	500 copies/mL	1 h	100 (1−2×)	100
Xpert Xpress SARS-CoV-2 test	Cepheid	250 copies/mL	<1 h	100 (2×)	100
Accula SARS-CoV-2 test	Mesa Biotech	100 copies/reaction	<1 h	100 (2−50×)	100
NeuMoDx SARS-CoV-2 Assay	NeuMoDx	150 copies/mL	<1 h	100 (1.5×)	100
ID NOW COVID-19 testa	Abbott	125 copies/mL	<1 h	100% (2−5×)	100
Biofire COVID-19 test	BioMerieux-BioFire Defense	330 copies/mL	<1 h	100 (1×)	100
Gnomegen COVID-19 RT-Digital PCR Detection Kit	Gnomegen	60 copies/mL	-	100 (1−2×)	100
		Serological Tests			
Platelia SARS-CoV-2 Total Ab assay	Bio-Rad		2 h	92.2	99.6
VITROS Immunodiagnostic Products Anti-SARS-CoV-2 IgG	Ortho-Clinical Diagnostics, Inc.		48 min	87.5	100
LIAISON SARS-CoV-2 S1/S2 IgG	DiaSorin Inc.		35 min	97	98
SARS-CoV-2 IgG	Abbott Laboratories Inc.		29 min	100	99.9
Elecsys Anti-SARS-CoV-2	Roche		18 min	100	99.8
Roche's Elecsys IL-6	Roche Diagnostics		18 min	84	63
Cellex qSARS-CoV-2 IgG/IgM Rapid Test	Cellex		15−20 min	93.8	95.6
Anti-SARS-CoV-2 Rapid Test	Autobio Diagnostics Co. Ltd. (jointly with Hardy Diagnostics)		15 min	99	99
COVID-19 Antibody Rapid Detection Kit	Healgen Scientific LLC		10 min	96.7	97
SARS-CoV-2 Total Assay	Siemens Healthcare Diagnostics Inc.		10 min	100	99.8
COVID-19 ELISA IgG Antibody Test	Mount Sinai Laboratory		<1 h	92.5	100
Anti-SARS-CoV-2 ELISA IgA and IgG	Euroimmun AG		-	90	100
New York SARS-CoV Microsphere Immunoassay	Wadsworth Center, New York State Department of Health		-	88	98.8
Vibrant COVID-19 Ab assay	Vibrant America Clinical Laboratories		-	98.1	98.6
RightSign COVID-19 IgG/IgM Rapid Test Cassette	Hangzhou Biotest Biotech Co., Ltd.		-	92.5	99.5

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Table 1. continued

product	company	LOD	time	sensitivity% (LOD dilution)	specificity%
		Serological Tests			
SCoV-2 Detect IgG ELISA	InBios International, Inc.		-	97.8	98.9

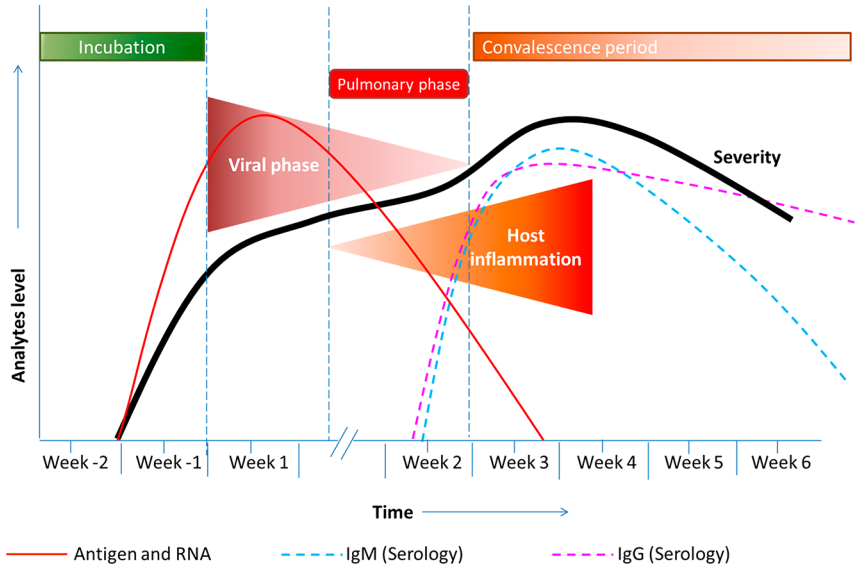

Figure 3. Standard relationship between the changes in analyte level with respect to the course of infection. In general, PCR tests are likely positive in the first week of infection, and likely negative after 3 weeks of infection due to overcoming the viral phase with the host response. This trend might vary from person to person. Adapted with permission from ref 55.

3. DIAGNOSIS AND CURRENT TREATMENT OPTIONS

It is highly difficult to differentiate the COVID-19 using common clinical tests from other respiratory disorders (influenza, respiratory syncytial virus (RSV), human metapneumovirus, other coronaviruses, bacterial, mycoplasma, and chlamydia infections).[38] The patients' average age for COVID-19 infection is much higher than that of MERS and Swine-origin influenza A (H1N1).[39,40] Most of the COVID-19 patients suffer from chronic comorbidities and undergo silent hypoxemia, i.e., no symptoms of respiratory failure. Some patients have failure of other vital organs, kidney, liver, and lungs, before respiratory failure. Thus, the new early warning score and quick sequential organ failure assessment may not help in foreseeing the respiratory failure.[41]

3.1. Diagnosis. The impact of early detection offers prevention of potential cases, while delayed reporting leads to minimal control of contagious diseases.[42−44] Currently, there are a number of nucleic acid- and protein-based diagnostic tests available (Table 1). However, there is also an urgent need for point of care (POC) devices for rapid, cost-effective, and self-diagnosis.

3.1.1. Nucleic Acid-Based Diagnosis Tests. Nucleic acid-based diagnostic tests are the most common for SARS-CoV-2 detection. Isothermal techniques require no specialized equipment, like polymerase chain reaction (PCR). Reverse transcription loop-mediated isothermal amplification (LAMP) based tests use the strand displacement polymerase instead of heat to generate the single strand template.[45−47] It uses the 4−6 primers and DNA polymerase, which binds to different regions of the target genome, and the amplified DNA is detected by the change in turbidity, which can be reflected in the change in color due to change in pH or fluorescent dye incorporated double-stranded DNA. The reaction completes in less than 1 h at 60−65 °C with ∼75 copies that can be easily detected.[48] This technique is very specific because of the usage of a high number of primers; it is simple, easy to visualize, has less noise, and does not require a thermocycler.[49] It can also be multiplexed at the reading or amplification stage by functionalizing the beads with different optical signatures or involvement of different genes to cut out the possibility of mutation leading to false negative tests.[44,48] The multiplexing helps to increase the information, specificity, and sensitivity of the tests. However, the challenges of LAMP tests are optimization of conditions and primers.

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-based Specific High-sensitivity Enzymatic Reporter un-LOCKing (SHERLOCK) diagnostic technology test became famous during the Zika outbreak, and could also be used for SARS-CoV-2 detection. Herein, the cDNA is synthesized and amplified using isothermal reaction, and then it again converted to RNA. Cas13a binds to amplified RNA, and on specific target binding gets activated, which then cuts off the quencher from fluorescence to give the signals.[50,51] It has been reported to detect ∼2000 copies/mL from serum and urine of Zika infected patients.[52] The optimized protocol for SARS-CoV-2 has been published with different Cas13a detection systems and soon might be commercialized.[53]

3.1.2. Protein-Based Tests. For the protein-based tests, antigens and antibodies are used for diagnosis. However, changes in viral load during the course of infection cause difficulties in detection, e.g., a high viral load in the salivary gland during the first week of infection that later declines[54,55] (Figure 3).

Antibodies may provide a longer window for detection; however, cross-reactivity of the antibody with other coronavirus antibodies poses a problem.[56] Currently, these serological tests

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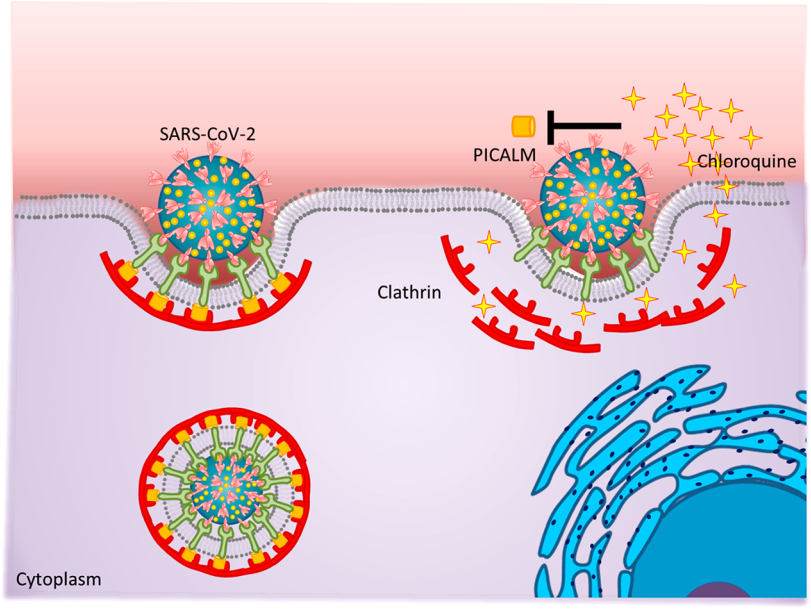

Figure 4. Potential mechanism of action of chloroquine against SARS-CoV-2. Chloroquine suppresses the expression of PICALM to inhibit the uptake of virus. PICALM is a clathrin assembly protein to assist the uptake of particles. Adapted with permission from ref 70.

are in development to detect asymptomatic patients.[57−59] The nucleocapsid protein is a highly immunogenic phosphoprotein and the most abundant in the virus. It is commonly used for the biomarker tests because it hardly mutates. Zhang et al. have used the Rp3 nucleocapsid protein from SARS-CoV-2 to detect the IgG and IgM antibodies from COVID-19 patients.[57] Rp3 nucleocapsid has been found to have 90% similarity with SARS related viruses. The serological tests are performed by adsorbing recombinant protein to the bottom of the multiwell plate, and then diluted patient serum is added to perform ELISA. The horseradish peroxidase functionalized secondary IgG anti-human antibody is added to obtain the signals. If anti-SARS-CoV-2 IgG is present, it will be sandwiched between the adsorbed protein and the anti-human IgG probe. Similarly, IgM has also been used for the sandwiched assay. It was found that after 5 days of symptoms, antibody level is increased. Like, on day 0 of SARS-CoV-2 infection, only 51% and 81% of patients were found positive for the IgM and IgG, respectively, and after 5 days, it increased to 81% and 100%, respectively. These antibodies may also be found in the suspected cases, as recent studies show other proteins and biomarkers may also be used for detection.[58] Currently, a number of serological tests have been approved by the FDA for the emergency use authorization (EUA) (Table 1).

3.1.3. Lymphopenia-Based Assessment. It has been observed that the course of the disease was different in different patients, but the variations in blood test parameters were constant from the onset of disease to death or discharge. The lymphocyte percentage was found to be the most consistent and significant parameter for the disease progress.[60] For example, in the sample size of 12 patients (death case) with the mean average age of 76, the lymphocyte percentage was reduced up to 5% in 2 weeks after the disease onset. In the case of 7 patients (average age 35, therapeutic time 35 days) with severe symptoms, the lymphocyte percentage decreased initially but increased to more than 10% when discharged; however, in 11 patients (average age 49, therapeutic window 26 days) with moderate symptoms, the lymphocyte percentage was almost constant and higher than 20% upon discharge. It shows lymphopenia to be one of the reliable parameters for determining the prognosis. The clinical data were collected from the General Hospital of Central Theater Command, Wuhan, China.[60]

3.2. Current Treatment Options. *3.2.1. Repurposable Drugs.* The repurposing of the drugs that have been approved for other indications has come up as an effective strategy to reduce the cost, time, and risk significantly in comparison to *de novo* drug development for COVID-19.

3.2.1.1. Chloroquine. Since 1934, the 9-aminoquinoline, known as chloroquine (CQ), has been an inexpensive and safe drug for the treatment of malaria and a prophylactic measure for autoimmune diseases. Recently, it has also been explored during *in vitro* studies for the antiviral properties against HCoV-OC43 and SARS-CoV.[61] It has been found to interfere with the ACE2 of the host cells and prevent the interaction of spike proteins of the SARS family of coronavirus.[62] However, the mechanism of action is still speculative. The determination of the precise mechanism is important for finding new prophylactic and therapeutic measures. The correlated studies show that chloroquine interferes with the pH-dependent viral fusion and replication, and also glycosylation of the receptor as well as envelope protein.[61,63] Additionally, it also interrupts the assembly in the endoplasmic reticulum−Golgi intermediate compartment. Chloroquine also attenuates the expression of proinflammatory receptors and factors mainly responsible for the SARS-CoV-2-associated mortality.[61,64]

SARS-CoV-2, similar to synthetic nanoparticles, falls in the same range of shape and size.[65,66] Thus, another mechanism is the suppression of phosphatidylinositol binding clathrin assembly protein (PICALM) for preventing the uptake of nanosized clathrin-mediated endocytosis[67−70] (Figure 4). This clathrin-mediated endocytosis path is being used by other members of the Coronaviridae family for entering the host cells. In the case of SARS-CoV, it is known that after binding to ACE2, endocytosis-driven entry of the virus is triggered.[71,72]

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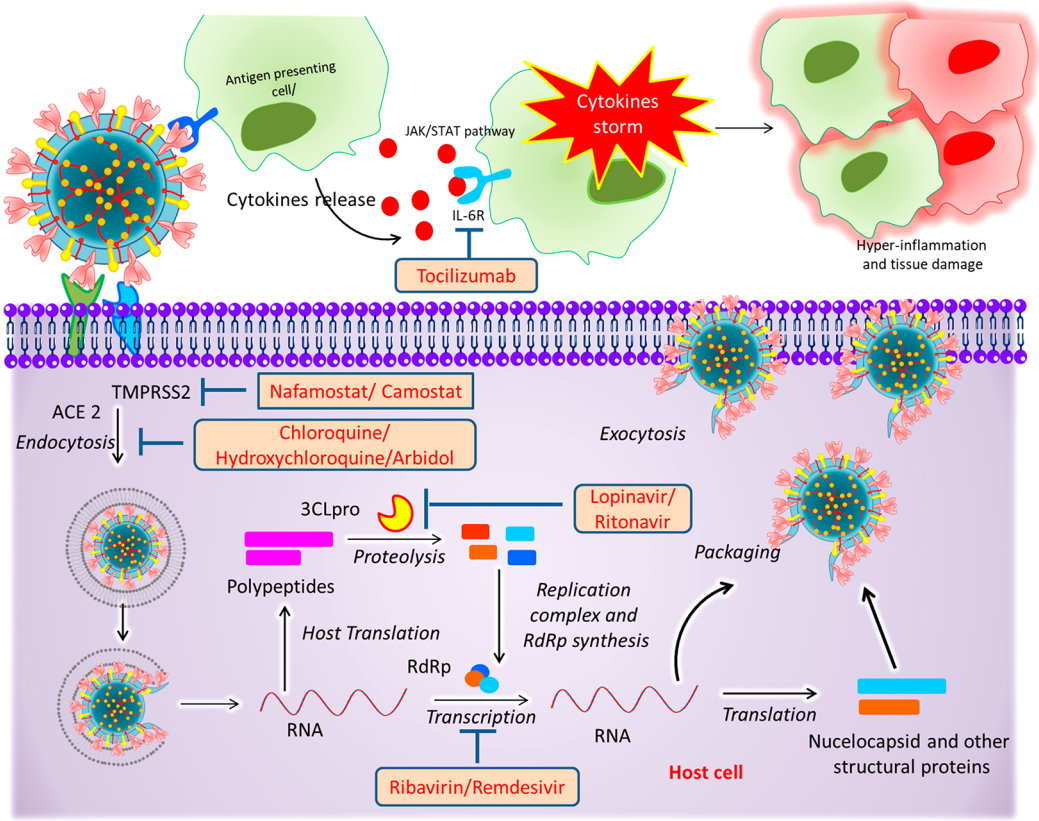

Figure 5. Mechanism of action of small molecules, antivirals, and protease inhibitors against SARS-CoV-2. Abbreviation: RNA-dependent RNA polymerase (RdRP).

Moreover, chloroquine prevents the fusion of endosomes with the lysosome to disturb endocytic trafficking.[70] It disrupts the membrane recycling, and thus the entry of viruses. The pH change in endosome for the activation of endosomal protease like cathepsin required for priming of S protein can be disturbed due to chloroquine driven inhibition of endosomal acidification, which leads to stalling of the virus in the endosome. Thus, there are chances of multiple mechanisms of action that are reflected in the broad-spectrum nature of the drug. Hydroxychloroquine (HCQ)has been reported to have the same anti-SARS-CoV-2 activity along with a higher safety profile.

The FDA suggests that excessive and long-term usage of CQ and HCQ result in eye damage, corrected QT prolongation, neuropsychiatric effects, and hypoglycemia.[73] These risks are amplified several-fold when CQ and HCQ are combined with other drugs (e.g., azithromycin) reportedly prolonging the QT intervals. Furthermore, heart problems are amplified if the patient is also suffering from renal diseases. Also, a paper published in the reputed journal *Lancet* corroborated the ineffectiveness of CQ and HCQ either with or without the combination of macrolide in the treatment of COVID-19, and also concluded that it might cause ventricular arrhythmias in the hospitalized patients. All these were indicated to revoke the CQ and HCQ EUA.[74] This report also halted the enrollment of patients in the WHO Solidarity clinical trial arm and other random clinical trials in different countries. However, shortly after a few weeks, this paper was retracted due to conflict between the coauthors due to the veracity of data sources. Meanwhile, a study published in another reputed journal, *New England Journal of Medicine*, again established that CQ and HCQ are no better than a placebo in the treatment of COVID-19 hospitalized patients. However, no comments were made on the usage for pre-exposure prophylaxis, which salvaged the continuation of clinical trials in high-risk populations.[75]

3.2.1.2. Ribavirin. Ribavirin (tribavirin) is an antiviral medication, a synthetic guanosine analog, widely used to treat hepatitis C, RSV infection, and viral hemorrhagic fever(s). It checks the hepatitis C virus polymerase and shows broad-spectrum effects against other DNA and RNA viruses (Figure 5). This drug was extensively used with or without steroids during the SARS outbreak.[76] The higher dose (1.2 to 1.4 g orally in every 8 h) for its effective action poses severe toxicities, e.g., hemolysis and liver injury.[77,78] Its inhalation formulation has also shown no benefits over oral and intravenous administration. Clinical studies showed that ribavirin could work synergistically with interferon β and inhibits SARS-CoV replication. However, blood transfusion was required in 40% of the patients.[79] Ribavirin shows teratogenic (abnormalities of physiological development) activity, and is thus not recommended for pregnant women.[80]

3.2.1.3. Lopinavir, Ritonavir, and Nelfinavir. Lopinavir (LPV) and ritonavir (RTV) are protease inhibitors widely used for the treatment of human immunodeficiency virus (HIV) (Figure 5). RTV is combined with LPV to increase the half-life by inhibiting cytochrome P450.[81] The LPV/RTV has been combined with ribavirin, which has shown better outcomes in the treatment of SARS.[82] Combination therapy using a cocktail of interferon-β, LPV/RTV, and ribavirin has been employed for the treatment of MERS.[83] This combination therapy is effective within 7−10 days of infection. The known side-effects of this treatment include diarrhea, nausea, and hepatotoxicity. This treatment may pose exacerbated effects in the case of COVID-

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19 patients (who may already be experiencing liver issues). It is important to note that HIV and SARS-CoV have different proteases; thus, there is also concern about the specificity of the combination.[70] Nelfinavir is a protease inhibitor of HIV, responsible for post-translational processing.[84] It strongly inhibits the replication of SARS-CoV, and is thus an option for SARS-CoV-2.[85]

3.2.1.4. Remedesivir. Remedesivir (GS-5734) is a monophosphate prodrug of an adenosine nucleoside analog. Remedesivir has been reported to inhibit the human and zoonotic coronavirus, including SARS-CoV in *in vitro* and *in vivo* studies[86] (Figure 5). This molecule shows superior activity (low EC_{50}) and selectivity of host polymerase against the Ebola virus. Remedesivir has been found effective in preventing lung hemorrhage during a murine MERS infection lung model. In the case of SAR-CoV-2, the *in vitro* studies have shown the EC_{50} to 0.77 μM and EC_{90} to 1.76 μM.[87,88] In recent reports, the combination of remedesivir and interferon-β was found to have higher efficacy than the triple combination of LPV/RPV, ribavirin, and interferon-β.[105] In the United States, the first COVID-19 patient was treated with remedesivir when the patient's condition was critical.[89] The random and controlled clinical trials of remedesivir are in process, and results have started to come. Remedesivir has been approved by the FDA for emergency use after finding that it reduced the hospitalization stay of COVID-19 patients.

3.2.1.5. Umifenovir. Umifenovir (Arbidol, ABD) is a small indole derived molecule for the treatment of respiratory viral infections, influenza, and prophylaxis in Russia and China.[90,91] The action mechanism includes interrupting the interaction of S protein and ACE2 receptor to inhibit the fusion of the virus to the host cell membrane[92] (Figure 5). ABD shows broad-spectrum antiviral activity by blocking the fusion of influenza A, B, and hepatitis C. The mesylate derivative of Arbidol has been found to be 5 times more effective than Arbidol in the treatment of SARS-CoV (*in vitro*).[93] A clinical trial data of 67 patients treated with Arbidol showed less mortality and a higher discharge rate compared to untreated patients.[94] However, further studies need to be conducted to reach any conclusion.

3.2.1.6. Nitric Oxide. Nitric oxide (NO) is a gas produced by NO synthetase and arginine. Nitric oxide changes to peroxynitrite by reacting with superoxides, and inhibits bactericidal and other cytotoxic reactions.[95] NO is given to manage the airway function and also used as an antiviral agent to inhibit the replication of RNA and protein synthesis of the virus.[96,97] The *S*-nitroso-*N*-acetyl penicillamine (NO-donor) significantly blocks the replication of SARS-CoV in a concentration-dependent manner.[98] Thus, NO inhalation is being considered as another choice for the treatment of COVID-19.

3.2.1.7. Dexamethasone. Recently, it has been reported that corticosteroid dexamethasone reduces the mortality of COVID-19 patients by 30%.[99,100] The higher mortality rate is primarily due to the hyperimmune response stemming from the inflammatory cytokine storm. Dexamethasone is an FDA-approved corticosteroid for immunosuppression, and 30 times more potent than cortisone. Although it inhibits the cytokine storm, also affects the T cells' adaptability and B cells' antibody-forming capability that could lead to persistently higher viral load even after the patient appears recovered. The function of macrophages required for blood clearance may be severely affected. Thus, it should be carefully used only for critically intubated patients and not for already recovering patients. It could be beneficial to give the combination of corticosteroid with natural flavonoids like luteolin known to have antiviral properties via nebulization for targeted delivery to the lungs.

3.2.2. Protease Inhibitors. 3-Chymotrypsin-like (3CL) protease, papain-like protease (PLP), and TMPRSS2 are the main proteases of coronavirus and responsible for the replication and inhibiting the host immune response.[101] Serine protease is mainly responsible for S priming and uptake of the virus. Thus, targeting of these proteases has been sought as a promising approach in controlling the coronavirus.

Camostat mesylate (CM), an inhibitor of TMPRSS2, can block the spread and pathogenesis of SARS-CoV and MERS-CoV (Figure 5). It is natively used to treat chronic pancreatitis in Japan. It prevents the uptake of SARS-CoV-2 by the ACE2 receptor. Currently, CM is in phase 2 clinical trial in the US. Cinanserin is a serotonin receptor antagonist and a very old drug used to inhibit the 3CL protease and thus replication of SARS-CoV.[30] The SARS-CoV-2 is also found to produce 3CL protease; thus, cinanserin could be another choice in controlling the pandemic.

Flavonoids are natural antioxidants and have several other medicinal effects, including antiviral activity. Flavonoids from *Pterogyne nitens* inhibit the infection of hepatitis C.[102] The other flavonoids, such as herbacetin, pectolinarin, rhoifolin, and biflanoids, have been tested for the inhibition of 3CL protease.[103,104] Quercetin 3-β-D-glucoside, herbacetin, and isobavachalcone have been found effective against the MERS-CoV. Diarylheptanoids (an extract from the bark of *Alnus japonica*) has been shown to inhibit the PLP of SARS-CoV very effectively.[105] Thus, the combination of cinanserin, flavonoids, and others could also be chosen as an alternative to fight against the SARS-CoV-2.

3.2.3. Biologics. The biologics have broadened the treatment options by leveraging the clinical practices during the SARS-CoV and MERS-CoV outbreaks. More than 500 patents have been filed featuring the antibodies, RNA, cytokines, vaccines, and other biologics.[106]

3.2.3.1. Antibodies. The neutralizing antibodies are the most common biologics. The literature suggests that the S protein of SARS-CoV-2 has been the target for antibody development due to its role in the fusion and uptake of the virus into the host cells.[107] The site saturation mutagenesis, along with the human framework reassembly and DNA display technology, is being used to determine the complementary regions of potent humanized mouse antibodies with high affinities. The antibody (2978/10) with more than 80% neutrality has been selected, which specifically targets the fusion region of the S protein. Therefore, understanding the additional details about this region is critical for the development of efficient antibodies.[108]

3.2.3.2. Cytokines. Low-molecular-weight biologics with immune-modulating properties can be generated to attack pathogens.[106] For ~40 years, different types of cytokines (chemokines, interleukins, interferons, and lymphokines) have been recognized. Interferons are the most common ones used to regulate the viral load by restricting replication. Interferon α and β have been studied for the coronaviruses, and interferon β has also shown a positive effect against MERS-CoV.[109] The combination with LPV/RTV and ribavirin can be expected to restrict the SARS-CoV-2 growth.

3.2.3.3. RNA Therapy. Complementary RNA neutralizes the mRNA and stops its expression. The interfering RNA is 21−25-nucleotides-long small interfering (siRNA) or micro RNA. The short hairpin RNA (shRNA) can be the precursors of siRNA,

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Table 2. Current Vaccines Undergoing Clinical Trials for COVID-19

vaccine, developer, platform, and stage of evaluation	clinical trial information
mRNA-1273, Moderna/Lonza, RNA, and Phase I	https://clinicaltrials.gov/ct2/show/NCT04283461
Ad5-nCoV, CanSino Bio, Non-Replicating Viral Vector, and Phase I	https://clinicaltrials.gov/ct2/show/NCT04341389
ChAdOx nCoV-19, University of Oxford, Non-Replicating Viral Vector and Phase I/II	https://www.clinicaltrials.gov/ct2/show/NCT04324606
LV-SMENP-DC, ShenZhen Geno-Immune Medical Institute, Lentiviral, and Phase I/II	https://www.clinicaltrials.gov/ct2/show/NCT04276896
BNT162 (a1, b1, b2, c2), (BioNTech, Fosun Pharma, Pfizer), RNA, and Phase I/II	https://www.clinicaltrials.gov/ct2/show/NCT04368728
New COVID-19 vaccine, Sinovac Biotech, Chemically Inactivated SARS-CoV-2, and Phase I/II	https://www.clinicaltrials.gov/ct2/show/NCT04352608
INO-4800, Inovio Pharmaceuticals, DNA, and Phase I/II	https://clinicaltrials.gov/ct2/show/NCT04336410
bac TRL-Spike, Symvivo Corporation, DNA, and Phase I	https://clinicaltrials.gov/ct2/show/NCT04334980
New COVID-19 vaccine, Beijing Institute of Biological Products, inactivated SARS-CoV-2, and Phase I	http://www.chictr.org.cn/showprojen.aspx?proj=52227
NVX-CoV2373, Novavax, Protein, and Phase I	https://clinicaltrials.gov/ct2/show/NCT03293498
Bacillus Calmette–Guérin (BCG) Vaccine, Research Group Netherlands, Live Attenuated Virus, and Phase I/II	https://clinicaltrials.gov/ct2/show/NCT04362124

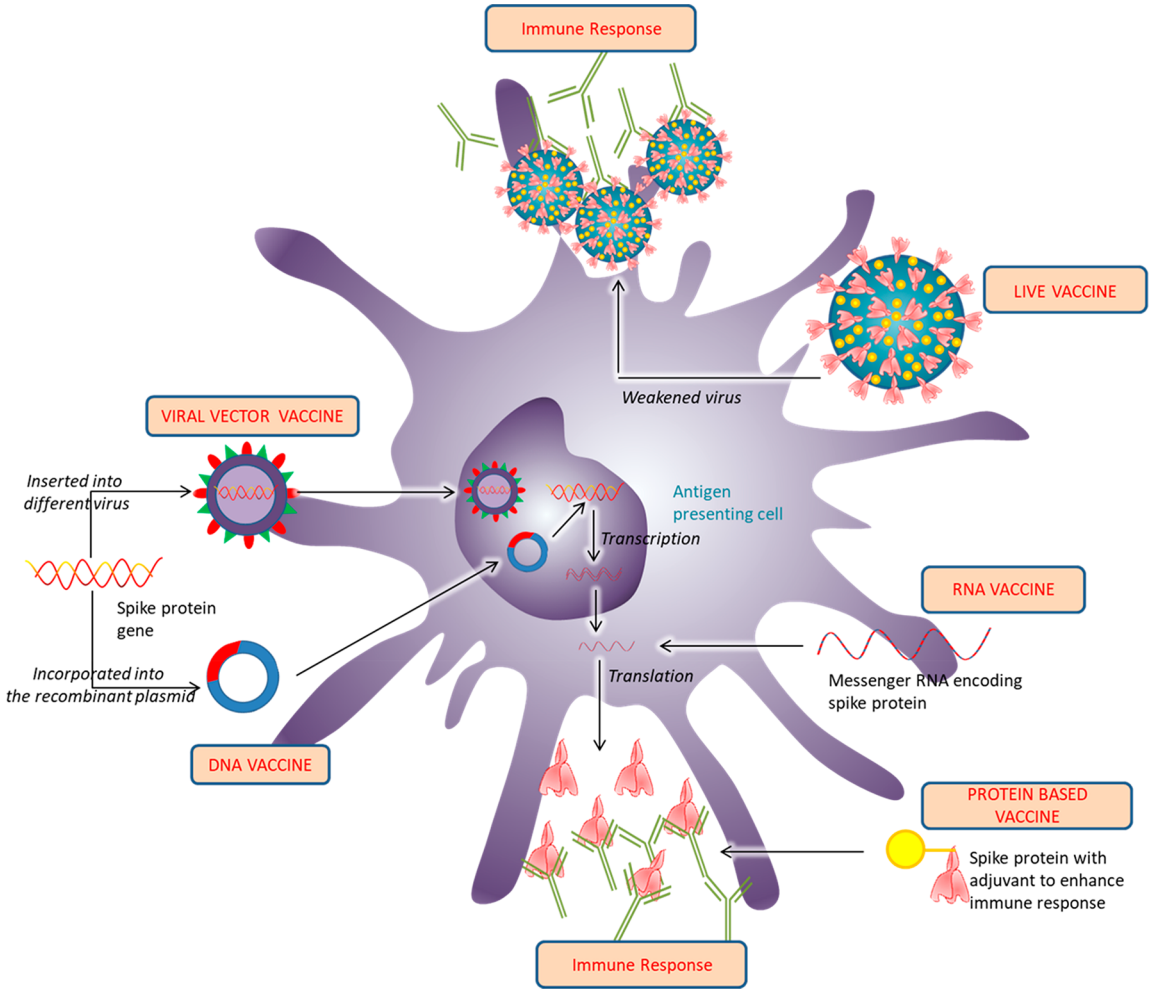

Figure 6. Approaches followed in the development of live, viral vector, DNA, RNA, and protein-based vaccine for SARS-CoV-2. Currently, most of the vaccines are in the clinical phase. Adenovirus-based vaccine (Ad5-nCoV) has been approved for military usage, and RNA-based vaccine (mRNA-1273) is gearing up for phase III clinical trial.

which folds into the hairpin structure and blocks the target gene. The antisense RNA is single-stranded, 19–23 nucleotides long, and occurs naturally or is synthesized. The delivery of interfering RNA is usually performed using viruses, bacteria, or delivery vectors like plasmids, liposomes, etc.[110]

Patent CN1648249 presents that dsRNA targets the M protein region of the mRNA from SARS-CoV. siRNA-M1 targets the 220–241 region of the M-mRNA, while siRNA-M2 targets the 460–480 region of the M-mRNA. The interference efficiency is more than 70%. The N and E region targeted siRNA can also inhibit the expression from GFP N and GFP E

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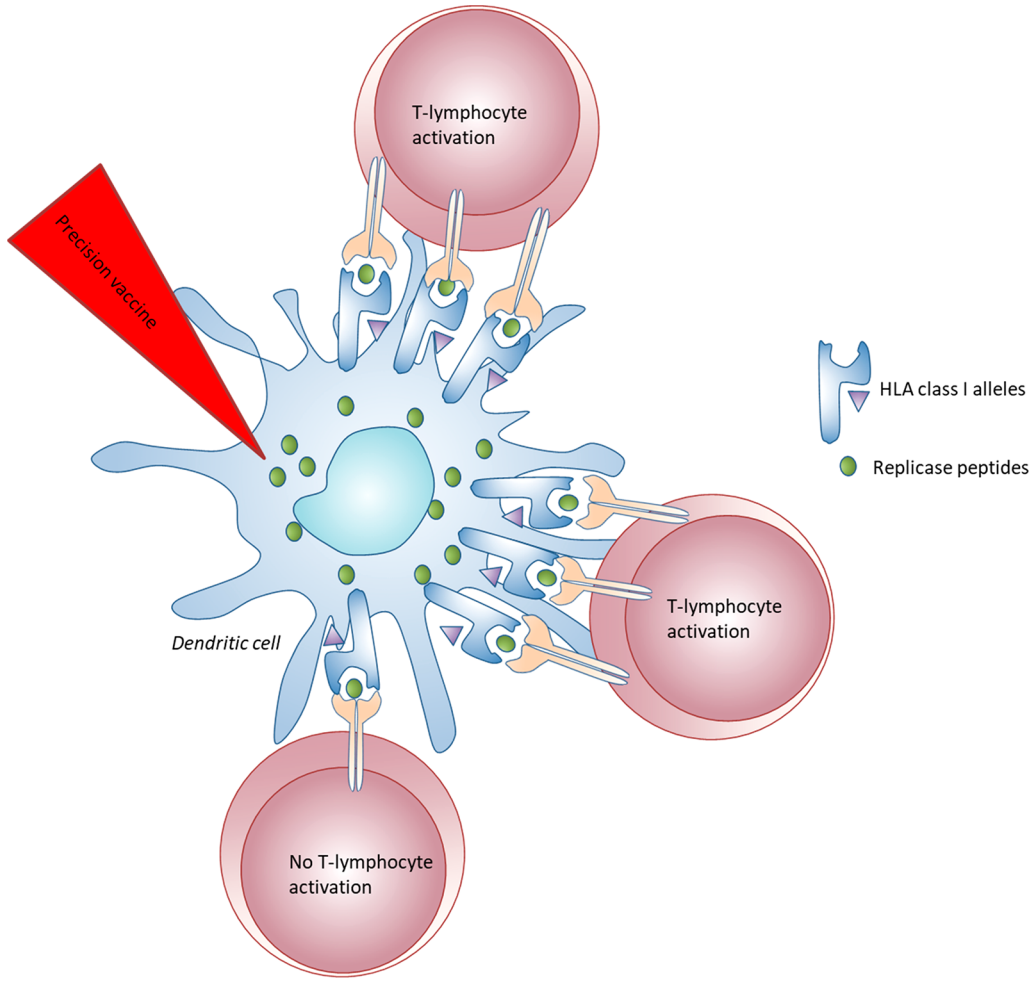

Figure 7. Role of precision vaccine in activating the T-lymphocytes. The peptide that binds 3 HLA class I alleles can activate the CTLs with more than 80% probability.

fusion proteins of SARS-CoV, respectively. The mutation at the 3′ end of siRNA increased the inhibition efficiency. The 6 siRNAs have also been proven to inhibit the replication by targeting the replicase 1A region of the SARS genome.[111] SARSsi-4 was found to be very efficient. There are also other siRNAs, e.g., SARSi-7, 8, 9, 10, and 11, which can target the S, M, N, and E region as disclosed by patent US20050004063A1. The efficiency of SARSi-2, 3, 4, and 7−11 has been tested in FRhk-4 cells and found to kill 50−90% of the virus.

The usage of RNA aptamers for SARS-CoV inhibition is also under an evaluation phase. RNA aptamers have a distinct affinity for the SARS-CoV nucleocapsid and inhibit the unwinding of DNA double helix. Patent JP2007043942 relates the development of ribozymes (RNA/DNA hybrid structure), which can recognize the conserved domain and loop region of the genes of coronavirus. It specifically recognizes the GUC having loop confirmation. Additionally, patent US20050004063A1 relates that the antisense oligonucleotide has been tested to reduce the severity of the coronavirus. DNA/RNA antisense has been designed for disrupting the pseudoknots in the frameshift region of SARS-CoV. It might also be used to target the proteins involved in the inflammation.

3.2.3.4. Vaccine. Currently, there are about 115 vaccine projects running around the globe. Out of these, 73 are in preclinical stages, and a few of them have moved to clinical trials like mRNA-1273, Ad5-nCoV, INO-4800, pathogen-specific aAPC, etc. (Table 2; https://clinicaltrials.gov, http://www.chictr.org.cn, Data accessed on May 18, 2020). Most of the vaccine development is in the USA (46%), while the rest are in China (18%), Asia (except China) and Australia (18%), and Europe (18%).[6]

Vaccines are broadly classified into the following types: attenuated, vector-based, protein-based, mRNA based, and DNA based vaccines[112] (Figure 6). Since the SARS-CoV-2 shares significant homology with SARS-CoV and MERS-CoV, analyzing the existing research could facilitate the development of vaccines for SARS-CoV-2. The safety of the vaccine is a very important factor along with its efficacy.[106] Almost half of the vaccine development was focused on the receptor-binding domain (RBD) of the S protein. It was found that there was a higher number of neutralizing bodies due to the recombinant S protein subunit with only 2-fold less than those produced by SARS-CoV.[113]

Attenuated Virus: After deleting the virulence determinant by reverse engineering, attenuated virus still remains the most robust and viable option for increasing immunity and wide cross-protection.[114] There are approaches like restricting the virus replication in the upper respiratory tract only with limited copy numbers. For example, mutations (Y6398H) at the Orf1a/b polyprotein inactivate (reduce the replication rate) the mouse coronavirus.[106] However, in the face of the pandemic,

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the large-scale production of attenuated virus in biosafety level 3 is a challenging task.

DNA Vaccine: The SARS antigen-specific CD8+ T cell response can be achieved with the help of chimeric nucleic acid, i.e., endoplasmic reticulum chaperone (e.g., calreticulin) linked to the antigen polypeptide of SARS-CoV.[115] Gold nanoparticles or other delivery vectors can further improve the delivery and the generation of humoral and T cell response specific to the nucleocapsid of SARS-CoV. It was observed that the titer of SARS was significantly reduced in vaccinated animals. DNA coated gold nanoparticles inducted both the humoral and cellular immune response with the help of IgG, neutralizing antibodies, and increase in T cell response (CD3+ CD4+ and CD3+ CD8+) for the release of TNF-α, IFN-γ, and IL-2.[116] Inovio Pharmaceutical announced that the DNA vaccine (INO-4800) is ready for SARS-CoV-2 and human trials to be initiated soon.[117]

Protein Vaccine: GlaxoSmithKline (GSK) is developing a protein-based vaccine with the help of S protein and oil-based adjuvant. The administration of soluble S protein (obtained from trimer-tag technology) along with GSK2 adjuvant produced high levels of neutralizing antibodies, and IgG2a and IgG2b antibody response in animal models.[118] Patent 20070003577 relates the usage of full-length recombinant trimeric spike protein of SARS-CoV for the generation of TriSpike vaccine. It showed the native immunogenicity comparable to the convalescent plasma of SARS patients. Also, a hybrid vaccine based on MHC II by Antigen Express in collaboration with Chinese consortium,[119] which consists of (i) an invariant chain, i.e., peptide enhancing the antigen presentation, (ii) linker between the peptide and antigenic epitope, and (iii) an antigenic epitope for the MHC II molecule.

microRNA Vaccine: Moderna has filed a patent (WO2017070626) for the development of the mRNA vaccine by combining the multiple mRNAs of S, S1, and S2 proteins of SARS-CoV and MERS-CoV into lipid nanoparticle formulation. This combination shows a very high level of neutralizing antibodies in comparison to the mRNA encoded by the S2 subunit. The viral load was reduced by 90% and the generation of the high amount of neutralizing bodies after its administration in New Zealand white rabbits. Moderna has announced the mRNA-1273 vaccine for SARS-CoV-2 clinical trials.[120] Currently, it has successfully completed the phase I and II clinical trials and is now gearing up for phase III clinical trials.[121]

The precision vaccines help in inducing the specific cytotoxic T-lymphocytes (CTLs) without generating the antibody response.[122] The clinical studies of SARS, MERS, and HIV have shown that the CTLs response protects patients from developing severe conditions due to a hyperimmune response. In the convalescent phase, the more severe the response, the higher the level of antibody was recorded in plasma.[123] Thus, the killing of infected cells with the help of CTLs in the absence of antibodies seems a better option. The CTLs induced by vaccines might kill the virus before it starts production. For example, CTLs should target the first polyprotein produced from the ORF lab of viral mRNA. The infected cell will process the polyprotein to peptides, and few of them will be presented on the surface of cells by the human leukocyte antigen (HLA) class I molecule, which could be recognized by CTLs. Hence, CTLs which are targeted to the replicase polyprotein will kill the infected cells even before it starts the synthesis of virus structural proteins.[124] It has been reported that CTLs can be induced with 80% probability by protein binding to more than 3 HLA class I molecule (Figure 7).

Topical delivery of DNA using polyethyleneimine-mannose nanoparticles induces a potent CTLs response in mice, monkeys, and humans.[125,126] Thus, a similar approach could be applied against SARS-CoV-2 by inhaling the particles and directing the CTLs in the lungs.[127]

3.2.4. Plasma Therapy. Due to the lack of vaccines and drugs, convalescent therapy/immunoglobulin therapy remains a potential option for severely ill patients.[128] The serum of recovered patients is used to treat the infected patients, as it contains the specific antibodies against the pathogen, which may clear the free virus as well as infected cells.[129] Convalescent plasma therapy reduces the virus titer in the acute phase and expedites the recovery process with the potential for preventing the reinfection.[130] The viral load in the blood is at its peak on the first week of infection, and hence is ideal for receiving convalescent plasma.[131] The collection of plasma globulin from the recovered patients remains the most viable option in the fight against the SARS-CoV-2; however, safety issues need further consideration. This therapy is more effective than the hormonal shock therapy used during the SARS and H1N1 pandemic.[132]

3.2.5. Nutritional Interventions. Vitamins also play a major role in the immune system. The deficiency of nutritional requirements is one of the reasons for the impaired immune response against infection.[133] For example, vitamin A plays a significant role in viral and bacterial infections.[134−136] Low vitamin A renders calves more susceptible for the bovine coronavirus.[137] Vitamin B functions as a coenzyme in many reactions.[138] Vitamin B and UV light have significantly reduced the titer dosage of MERS-CoV.[139] Additionally, vitamin B3 can also reduce the neutrophil infiltration to the lungs, and thus the inflammation. Vitamin C has also been reported to protect against coronavirus.[140] The chicken trachea organ culture demonstrates resistance to coronavirus after vitamin C supplement. It also helps in the production of histamines, which help in runny noses, sneezing, swollen nodes, and other flu-like symptoms.[141] Three clinical trials have shown the involvement of vitamin C in the prevention of pneumonia susceptibility.[142] Vitamin D is also presumed to have a significant role in COVID-19. Vitamin D deficiency makes calves prone to bovine coronavirus.[143] In addition to these vitamins, mucroporin-M1, emodin, promazine, and nicotinamide are also known to work against SARS-CoV and SARS-CoV-2.

4. NANOTHERANOSTICS PERSPECTIVE

There have been a number of nanotechnology inventions which were applied right from diagnosis, surgical and therapeutic interventions. Nanotechnology has emerged as a wonderful tool and technology to enhance the efficacy of existing therapeutic and imaging agents. A number of multifunctional nanoparticles have been developed and approved by the FDA for various indications, including cancer treatment. Bayer's aspirin is also a particle formulation for quick relief from pain. Now, there is a circumstance to tune already FDA-approved formulations for tackling COVID-19. This section delineates the nanotechnological advancements and resources that can be used for the diagnosis, prevention, and treatment of viral disease, and easily extrapolated for COVID-19.

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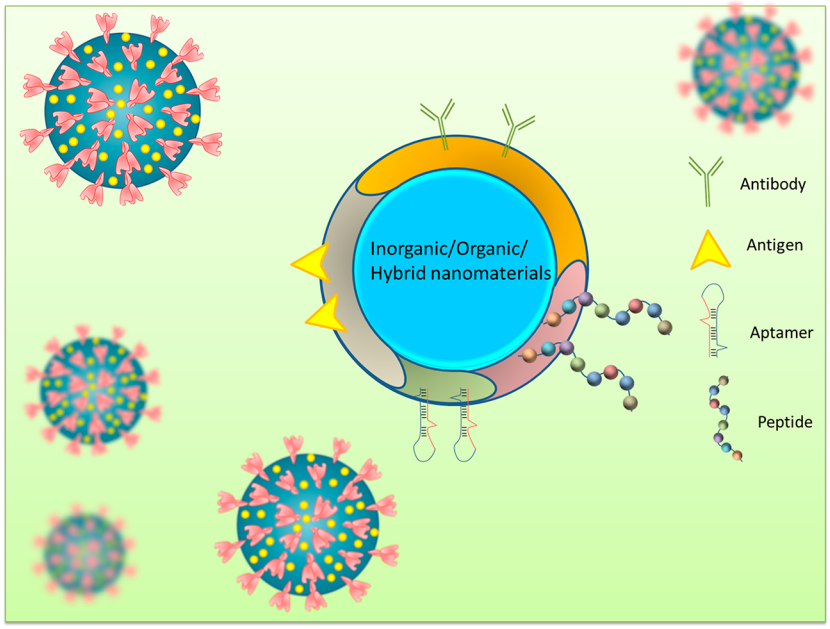

Figure 8. Role of inorganic, organic, and hybrid nanomaterials in COVID-19 theranostic. The antibody and antigen functionalization of nanomaterials is the most common approach for diagnostic purposes.

Table 3. Nanomaterials Based Diagnostic Tests Available for SARS-CoV-2 and Related Viruses[a]

type	target	virus	nanomaterial	role	LOD	ref
Piezoelectric immunosensor	Antigen (sputum)	SARS-CoV	Piezoelectric crystal consisted of quartz wafer	Immobilization of polyclonal antibodies against SARS-CoV	0.6 μg/mL	153
LSPCF	Nucleocapsid protein (serum)	SARS-CoV	Gold nanoparticles	Immobilization of fluorophore labeled anti-N-2 antibodies	1 pg/mL	154
Optical immunosensor	Antigen (nucleocapsid protein)	SARS-CoV	Quantum dots	Immobilization of RNA aptamers	0.1 pg/mL	155
Electrochemical immunosensor	Antigen	SARS-CoV-2	Gold nanoparticles	Immobilization of mAbs	90 fM	156
FET	Antigen	SARS-CoV-2	Graphene sheets	Immobilization of specific antibodies	1.6×10^1 pfu/mL	157
PPT effect and LSPR	RNA	SARS-CoV-2	Gold	Immobilization of DNA	0.22 pM	158
Electrochemical immunosensor	1-naphthol	Influenza	Pt/CeO_2/GO composites	Immobilization of antibodies and signal amplification	0.43 pg/mL	159
Electrochemical immunosensor	PB1-F2 protein	Influenza A	Polypyrrole matrix	Immobilization of monomeric or oligomeric PB1-F2 specific antibodies	0.42 nM	160
Nanoflow immunosensor	Antigen	H1N1, H5N1, and H7N9	ZnO nanorods grown inside PDMS channel	Immobilization of antibodies	1 pg/mL	161
LSV	Antigen	H7N9	AgNPs-G/AuNPs-G	AgNPs-G as trace labels/AuNPs-G for immobilization of H7-mAbs	1.6 pg/mL	162
Electrochemical immunosensor	Antigen	H7N9	Bifunctional magnetic nanobeads	Separation and signal carriers	6.8 pg/mL	163
Electrochemical immunosensor	Antigen	H1N1	RGO	Immobilization of mAbs	0.5 pfu/mL	164
SPR	Surface antigen	AIV	Gold nanoparticles	Immobilization of GBP/array chip	1 pg/mL	165
Fluorescence immunoassay	Antigen	AIV	Gold nanoparticles and quantum dots	Labeling and fluorescence quenching	0.09 ng/mL	166
Voltammetry	cDNA	AIV	MWNT, PPNWs, and gold nanoparticles	Immobilization of DNA aptamer	0.43 pM	167
FRET	cDNA	AIV	Quantum dots	Immobilization of oligonucleotides	0.27 nM	168

[a]Abbreviations: Graphene oxide and Pt nanoparticles functionalized CeO_2 nanocomposites (Pt/CeO2/GO), silver nanoparticle-graphene-chitosan nanocomposite (AgNPs-G) /gold nanoparticle-graphene nanocomposites (AuNPs-G), localized surface plasmon coupled fluorescence (LSPCF), linear sweep voltammetry (LSV), reduced graphene oxide (RGO), plasmonic photothermal (PPT), localized surface plasmon resonance (LSPR), fluorescence resonance energy transfer (FRET), polydimethylsiloxane (PDMS), monoclonal antibodies (mAbs), field-effect transistor (FET), gold binding polypeptides (GBP), multiwall carbon nanotubes (MWNT), polypyrrole nanowires (PPNWs), avian influenza A subtype (H7N9).

4.1. Detection. Biosensors are an alternative to enzyme-linked immunosorbent assay (ELISA) and have been developed for several viral diseases.[144] However, low sensitivity and specificity are a major concern. Nanotechnology plays an important role in biosensor(s) development. It facilitates the targeted sensing, multiple analyte detection, ability to mass

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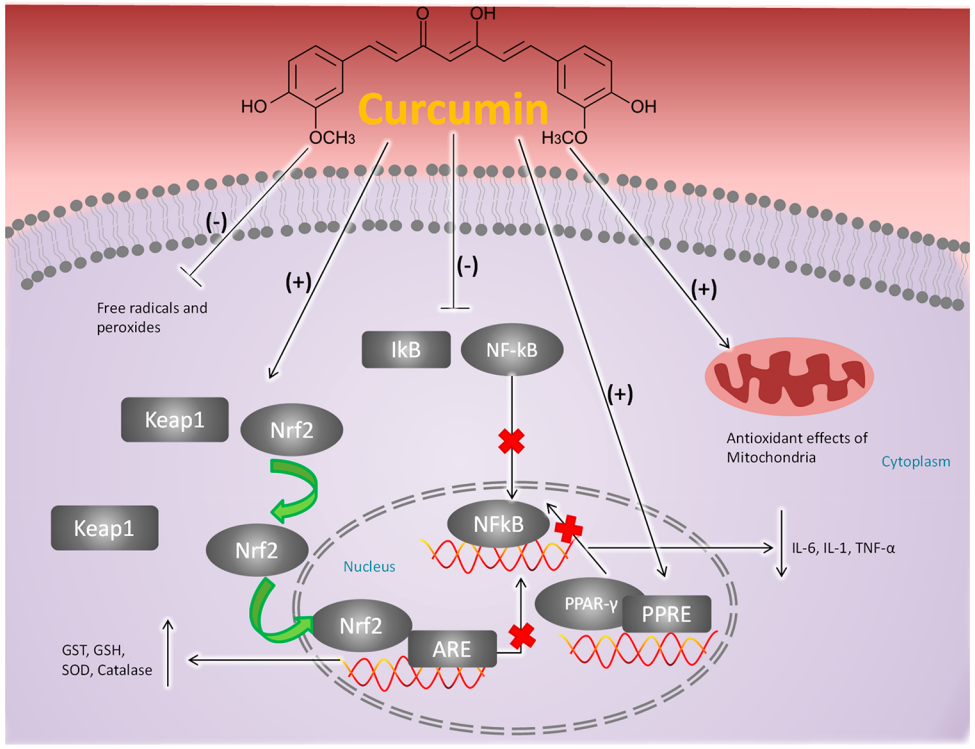

Figure 9. Anti-inflammatory action mechanism of curcumin. It follows diverse pathways to inhibit the inflammation. Abbreviation: Nuclear factor erythroid-derived 2(Nrf2), Kelch-like ECH-associated protein 1, antioxidant responsive element (ARE), glutathione S-transferase (GST), glutathione peroxidase (GSH), superoxide dismutase (SOD), peroxisome proliferator-acitvated receptor-γ (PPAR-γ), PPAR responsive elements (PPRE), nuclear factor-κB (NFκB), inhibitor of kinases (IκB Adapted with permission from ref 179.

transfer at short distances for fast response, etc.[8] This leads to high sensitivity, selectivity, and accelerated development of POC devices. Plasmonic nanoparticles, such as silver, gold, and other metal oxide nanoparticles, quantum dots, and graphene have gained significant interest in the field of biosensors (Figure 8).[9,145]

The intriguing optical property (surface plasmon resonance) of nanoparticles is being utilized to enhance the electromagnetic radiative phenomenon (absorbance and scattering) in diagnosis. The localized surface plasmonic resonance (LSPR), surface-enhanced Raman spectroscopy (SERS), quenching, and fluorescence properties are widely used in biosensor function. Gold nanoparticles (AuNPs) attached to the antibodies, biomolecules, proteins, and aptamers can enhance the LSPR and SERS signals, and the energy transfer between the fluorophore and AuNPs.[9,146] Currently, there are a number of nanomaterial-based biosensors available for the detection of SARS-CoV-2 and related viruses (Table 3).

For example, AuNPs deposition on the indium tin oxide (ITO) coated glass slides was capable of enhancing the detection of electron transfer signals from the HIV-1 virus-like particles (VLP).[147] This system detects HIV-1 at a concentration of 600 ng mL^{-1} to 375 pg mL^{-1}. Silver nanoparticles (AgNPs) are useful in increasing the intensity of electron transfer to the electrode surface. AgNPs are mostly exploited in the electrochemiluminescence (ECL) biosensors.[145] Functionalization and immobilization of AgNPs provide enhancement to the ECL of the luminophores. Nanosilver substrate has been found very promising for the SERS, which helps in achieving reproducible signals in aqueous solutions.[148] Moreover, the combination of Ag and Au has been used for the synthesis of bimetallic NPs for more efficient sensing applications.[149]

Silicon nanowires are emerging as an ultrasensitive and label-free detection technique. Silicon nanowire-based transistors are able to detect even the single virus after modification with antibodies.[150] On combining the air sampling with the microfluidics, it was noticed to detect even airborne influenza-like H3N2 viruses.[151] A surface plasmon resonance biosensor (without label or enzyme amplification) can detect the HIV-related DNA with high accuracy and reproducibility at a linear range of 1 pM to 150 nM and limit of detection (LOD) of 48 fM. The time required for the test is ~60 min.[152]

Nanozymes are the artificial enzymes comprising nanomaterials that have similar efficiency and catalytic abilities as natural enzymes.[169] For example, Fe_3O_4 nanoparticles exhibit intrinsic peroxidase activity, while many other nanomaterials have shown enzymatic activities such as catalase, oxidase, superoxide dismutase, and many others.[170,171] Nanozymes can also regulate the redox level of the cells, as the excess ROS causes oxidative stress and damages the cells.[172] Nanozymes have been used for detecting viruses, such as HIV and Ebola. A BSA-Ag nanocluster formulation can enhance Hg^{2+} oxidase activity, which can produce superoxide anions for the 3,3,5,5-tetramethylbenzidine (TMB) oxidation. This system was able to detect the 20 nmol L^{-1} of HIV DNA.[173] Another nanozyme system was found to be 100-fold more sensitive and quick in the detection of Ebola.[174] Together, it is viable that by combining different types of antibodies and probes, these diagnostic strips can be applied for different viruses.

4.2. Nanotherapeutics. *4.2.1. Nanomedicine.* The size and morphology of nanomaterials are comparable to the SARS-CoV-2, which can facilitate binding to the spike protein and help in the localized killing. There is an enormous demand for nanotherapeutics or nanomedicine (medicine in nanoparticles)

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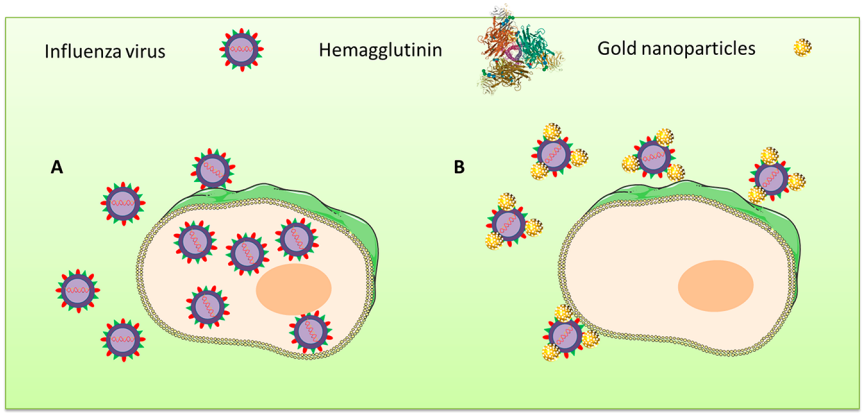

Figure 10. Mechanism of action of porous gold nanoparticles to prevent the attachment of viruses on the cell surface. A. Virus interaction with host cells and internalization through cellular receptors with hemagglutinin. Note: Hemagglutinin exists on the surface in cellular membranes. B. Prevention of cellular receptor and hemagglutinin binding via nanoparticles. The porous gold nanoparticles provide larger surface area for the breakage of disulfide bond. Adapted from ref 217.

for the treatment of various diseases. These nanoparticles can efficiently deliver previously mentioned numbers of repurposing and other therapeutic agents. However, in this section, we focused on two molecules, curcumin and niclosamide: how nanoformulations of these molecules can be improved for viral diseases, including SARS-CoV.

Curcumin is a polyphenol compound obtained from *Curcuma longa* and extensively used as an anti-inflammatory agent.[175,176] Thus, the usage of curcumin seems promising for the COVID-19 driven severe lung inflammation. Inflammation is the result of immune response, overexpressed inflammation markers, and increase in lipid peroxide and free radicals.[177] Curcumin efficiently interrupts various pathways, such as NF-κB, tumor necrosis factor-alpha (TNF-α), cyclin D1, signal transducer, cyclooxygenase, and transcription activators[178,179] (Figure 9). It also suppresses the expression of interleukin 6 (IL-6) by inhibiting the mitogen-activated protein kinase (MAPK) and NF-kB signaling pathway.[180,181]

Curcumin inhibits the Epstein−Barr virus by blocking the inducers and suppresses the proliferation of Epstein−Barr virus transformed human B cells.[182] It can obstruct the integrase 1 for preventing HIV infection.[183] Curcumin has been found effective for the treatment of various viral diseases such as chikungunya, hepatitis C, Japanese encephalitis virus (JEV), vesicular stomatitis virus (VSV), flock house virus (FHV), herpes simplex virus (HSV), feline infectious peritonitis virus (FIPV), and RSV.[184,185] It was observed that at 30 μM curcumin, the strains of HIN1, H6N1, and influenza A virus (PR8 strain) were inactivated, also the infection was reduced due to HSV-1 and HSV-2 virions in the Vero cells.[186] In addition, it has been tested in silico for the treatment of Ebola.[187] It also worked against the Zika virus in a dose- and time-dependent manner.[188]

Although curcumin has diverse medicinal properties, the low bioavailability limits its application. Various methods have been proposed to increase bioavailability, such as loading curcumin in polymer micelles, polymer nanoparticles, liposomes, metal complexes, and analogs, as well as derivatives.[189] There are several nanoformulations of curcumin, with the most common ones being poly(lactic-*co*-glycolic) acid, poly-(butylcyanoacrylate), chitosan, solid lipid, and albumin nanoparticles.[190] For example, the binding of curcumin with chitosan increases its bioavailability, biodistribution, and chemical stability and reduce systemic toxicity.[191] Curcumin nanoformulations have been shown to have minimum inhibitory concentration of ∼1 mg/mL while the crude one had 350 mg/mL.[192] It reflects the superior anti-pathogenic activity of curcumin when encapsulated in nanoparticles. The PEGylated lipid formulations of curcumin have been found to be 10-fold more effective for malaria than crude curcumin.[193,194] The composite nanofilms based on curcumin, chitosan, and silver nanoparticles or nanoclay have been reported to induce synergistic antibacterial effects.[195,196]

Niclosamide is an FDA-approved antihelmintic drug developed for the treatment of tapeworms and nematodes. Currently, WHO has listed it as one of the essential drugs.[197] Niclosamide inhibits oxidative phosphorylation and stimulates the ATPase activity in the mitochondria and regulate the other pathways, such as Wnt/β catenin, STAT3, Notch, mTORC1, NF-kB, NS2B-NS3, and pH in treatment of bacterial and viral infections, cancers, and other metabolic diseases.[198] Niclosamide inhibits the replication of SARS-CoV in less than 0.1 μM (EC_{50} value) along with cutting off the cytopathic effect at 1 μM.[199] Its 2-chloro-4-nitroanilide derivative is a potent inhibitor of 3CL protease of SARS-CoV. Niclosamide was found to be more effective at a concentration of 10 μM to MERS-CoV with 1000-fold reduction in replication by inhibiting S-phase kinase associate protein 2 (SKP2) through autophagy induction.[200] This drug has also been tried for the treatment of flavivirus, hepatitis C, Ebola, rhinovirus, chikungunya, human adenovirus, and Epstein-Barr virus.

However, niclosamide is also not spared from demerits, such as non-negligible cytotoxicity, high hydrophobicity, low bioavailability, and absorption. To overcome these limitations, a number of strategies, such as chemical modification, prodrug, and loading in drug carriers, have been implemented to improve the pharmacokinetic properties and to maximize the therapeutic versus side-effect ratio.[201,202] A nanocrystal suspension formulation of niclosamide (nebulizer, 1−5 μm droplet size) stabilized by the surfactant can deliver a therapeutic load to the lungs.[202] The pegylated nanoformulation has been proven to increase the hydrophilicity and half-life, enhance the bioavailability, and reduce the systemic toxicity.[203] Other methods are encapsulation inside albumin, solid lipid, and polymeric nanoparticles. In addition, it has been conjugated with the chimeric polypeptide, which self-

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assembles to form the monodisperse nanoparticles and worked as a prodrug for various types of infection.[204]

4.2.2. Inorganic Nanoparticles. The commercial antiviral drugs for the influenza virus mainly target neuraminidase (NA) and matrix protein 2 (M2) ion channel.[205] The M2 ion inhibitor blocks the transportation of H^+ ions, thus the coating of the viral genome, and prevents the replication. NA inhibitors prevent the release of virus from the cells by interrupting the hydrolysis of the terminal sialic acid residues. The US Center for Disease Control (CDC) says that 99% of influenza virus strains show resistance to M2 inhibitors.[206] The continuous mutations have changed the drug binding sites of the influenza virus, and thus increased the infectivity and mortality rates.[207,208] Another target is hemagglutinin (HA) (involved in the fusion of the virus with the host cells) of the influenza virus.[209] Silver nanoparticles interfere with the disulfide bonds of HA and block entry of the virus into host cells.[210] Additionally, there have been many combinations of silver and gold nanoparticles, such as tannic acid, chitosan, poly(*N*-vinylpyrrolidone) (PVP) and curcumin-modified silver, and gallic acid-modified gold nanoparticles for checking the infection of influenza virus.[211−215] Porous gold nanoparticles provide extensive surface area and more efficiently bind the disulfide bonds[216] and stop the viral infection compared to conventional inorganic nanoparticles (Figure 10).[217]

The other perspective is to develop the inanimate surface by spraying the antiviral nanoparticles. This will inactivate the virus even before it attacks the body. These nanoparticles can release active ingredients on the surface in a slow and controlled manner. Zinc oxide (ZnO), copper, silver, and other cationic nanomaterials are being used for the development of self-cleanable cloths with broad antiviral activity.[218,219] There is a report that viral infections progress due to the deficiency of zinc; thus, being exposed to zinc due to nanocoating of inanimate surfaces might help another way.[220] There are established reports that SARS-CoV-2 spreads through the air in droplets, and it comes out when someone talks, sneezes, or even breathes heavily. In such conditions, the antiviral nanocoating on the mask can prevent such intermediate infection. Such coating approaches may desiccate the virions and reduce capillary condensation of water.[221] These nanocoatings can become active upon exhalation, i.e., in the presence of warm and moisturized air, but not in the cold and dried inhaled air. The implementation of nanocoatings can be widely applied without disrupting the supply chain of masks.[222] These coatings are already in use to prevent mold, mildew, and other humidity problems in buildings.[223]

4.2.3. Multifunctional Nanotherapy. The long-life dosage regimen of antiretroviral therapy (ART) causes pill fatigue. Thus, there is a requirement of long-acting theranostic solutions. Nanomaterials act as contrast agents or contrast enhancers in imaging applications. Bismuth- and sulfur-containing nanorods intrinsically radiolabeled with luteium[177] (^{177}LuBSNRs) can render both antiviral therapy and multimodal imaging.[224] The gold nanorods combined with the curcumin loaded poly (lactic-*co*-glycolic acid)-*block*-poly (ethylene glycol) (PLGA-b-PEG) copolymer are helpful for triggered and localized release of therapeutics along with photothermal capacity in one system.[225] The albumin-Bi_2S_3 and magnetic nanoparticles can be developed for the combination of radiotherapy, magnetic hyperthermia, and chemotherapy. In comparison to nontargeted nanoparticles, the retention time of targeted nanoparticles increases by 3−6-fold.[226] Several other inorganic, organic, and hybrid multifunctional nanoparticles can be applied for viral diseases (Table 4).

Table 4. List of Inorganic, Organic, and Hybrid Nanomaterials Used for Viral Disease Theranostic Therapy[a]

nanomaterials	virus	therapeutic potential	imaging potential
Silver nanoparticles	HHV, HIV-1, RSV, MPXV, IV, TV, HBV, CoV	Affect attachment and block replication	SERS
Gold nanoparticles	IV, HSV-1, CSFV, HIV	Prevent attachment as well as penetration, PTT	CT, PAT, SERS
Copper oxide nanoparticles	HSV-1, HIV-1	Degradation of genome and oxidation of proteins	PAT
Zinc oxide nanoparticles	H1N1, HSV	Inhibition after viral entry	Optical
Zirconia nanoparticles	H5N1	Promote cytokines	Optical, MRI
Silicon nanoparticles	IV	Reduce number of progeny	Optical
Nanocarbon	HHV, IV	Inhibit entry of virus, PTT	SERS, Optical
Graphene oxide	HHV, HCV, RSV	Inhibit attachment, PTT	SERS, Optical
Iron oxide nanoparticles	HCV	Magnetic hyperthermia	MRI
Dendrimers	HIV, HSV	Drug delivery system	Multimodal
Polymeric	HIV, IV	Drug delivery system	Multimodal
Biological nanoparticles	IV	Antigenic presentation, Drug delivery system	Multimodal
Liposomes	HCV, IV, HIV	Antigenic presentation, Drug delivery system	Multimodal

[a]Abbreviation: Human herpesvirus (HHV), human immunodeficiency virus type 1 (HIV-1), respiratory syncytial virus (RSV), monkeypox virus (MPXV), influenza virus (IV), Tacaribe virus (TV), hepatitis B virus (HBV), coronavirus (CoV), swine fever virus (CSFV), human papillomavirus (HPV), herpes simplex virus type 1 (HSV-1), herpes simplex virus (HSV), hepatitis C virus (HCV), avian influenza virus subtype (H5N1), surface-enhanced Raman scattering (SERS), computed tomography (CT), photoacoustic tomography (PAT), single-photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), photothermal therapy (PTT).

4.2.4. Nanovaccine. The majority of the vaccines help in generating antibodies for neutralizing the pathogen; however, recent findings show that activation of both humoral and cellular immune response is necessary to control diseases like HIV, hepatitis C, and many others.[227−229] Virus-like nanoparticles (VLPs) balance the safety and immunogenicity of the vaccine. VLPs are noninfectious and at the same time carry similar protein compositions to that of the virus. Most of the VLPs do not have nucleic acid; some have but do not allow it to be replicated in mammals. Currently, hepatitis B and E and human papillomavirus vaccines are based on VLPs.[230] These vaccines require the adjuvants to be completely effective. The VLP size ranges 20−300 nm, which can be efficiently recognized by dendritic or other antigen-presenting cells.[231,232] VLPs also help in generating the innate immune response with the help of pathogen-associated molecular patterns (PAMPs), leading to the generation of type I interferon (IFN-I), better cross-presentation capacity, and cell-mediated T lymphocyte response.[233−235] Further, VLPs have been studied to induce the cellular immune response, wherein antigen containing VLPs were presented by APCs to activate the pathogen-specific CD8+ T cells through a TAP-

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Table 5. Clinical Trials of VLPs for Respiratory Viruses[a]

source	component	expression system	protective immunity	age group
H1N1, H5N1[240]	HA+NA+M1	*E. coli*	Neutralizing antibody with 79% seroprotection rate	18−64
H5N1[241]	HA+NA+M1	*E. coli*	Seroconversion: HAI based—61% MN based—76%	18−40
H7N9[242,243]	HA VLP + ISCOMATRIX	*E. coli*	Neutralizing antibody against both homologous and heterologous strains (H7-A/Netherlands/219/03 strain)	≥18
H1N1[244]	gH1-Qbeta/alhydrogel	*E. coli*	Seroconverison − Adjuvant group: 51.2%; Nonadjuvant: 70.3	21−64
H5N1[245,246]	HA	*Nicotiana benthamiana*	Hemagglutination inhibition with virus microneutralization	18−60
RSV-A2[247,248]	RSV F	Sf9 insect cell	Neutralizing antibodies	>55 and healthy women

[a]Abbreviation: Swine-origin influenza A (H1N1), Avian influenza A subtype (H7N9), hemagglutinin (HA), neuraminidase (NA), matrix (M1), Globular head domain of hemagglutinin (gH1).

proteasome independent pathway.[236−238] VLPs are safer than conventional vaccines and can be injected into immunocompromised individuals.[239] There are a number of VLPs that have been developed for several viral infections (Table 5).

The Novovax patent (US20160206729A1) discloses that VLPs containing at least one trimer of S protein of MERS-CoV induced the high level of neutralizing antibodies in conjugation to proprietary adjuvant Matrix M in mice and cattle. These VLPs were proven to promote protection against the MERS-CoV. Recently, Novovax has announced that it is working with SARS-CoV-2 using patented recombinant technology and proprietary adjuvant.

4.2.5. Nanosponges. The nanosponge category of nanoparticles is a new generation of drug delivery system[249] and acts as virus filter(s). The unique structure and core of nanosponges can separate viruses. The exterior cross-connected structure with cavities (250−1 μm width) of nanosponge similar to RBCs can also release drugs at the site in a controlled manner.[250−253] Nanosponges can also act as depots for soaking up the toxins. For example, on the injection of nanosponges, bacteria and other pathogens attack to it, being similar to the RBCs. Once these depots are saturated with toxins, they go to the liver and filter out content.[253] Nanosponges have been used for the treatment of viral diseases like influenza, rhinovirus, RSV, HIV, and HSV.[254−256] Such systems with antivirals effectively inactivate and kill the viruses in nasal epithelial and lungs.[257]

4.2.6. Autophagy Modulation. Autophagy has received significant attention in the past decade since the outbreak of SARS in 2002. There is evidence suggesting the involvement of endocytic pathways in facilitating the entry of coronaviruses like SARS-CoV and MERS-CoV via two critical steps, i.e., (i) clathrin-dependent endocytosis and (ii) cathepsin-mediated S protein cleavage.[258] It has also been experienced that enhancing the autophagy of viral proteins heightened adaptive immunity due to an increase in antigen priming.[259] In addition, the role of autophagy has been established substantially in the regulation of inflammatory cytokines. Thus, the autophagy modulating drugs in combination with antivirals might be another breakthrough in handling the COVID-19 crisis. Further, a recent study also suggests that out of two synonymous mutations in SARS-CoV-2, nonstructural protein 6 (NSP6) mutation could lead to a significant change in the pathogenicity and intracellular survival of the SARS-CoV-2 due to its involvement in the autophagic lysosomal machinery.[260] Thus, there is also a need to monitor this mutation and leverage the opportunity.

In addition, evidence shows that nanomaterials can be tuned accordingly to modulate the endocytic pathway toward the treatment and control of diseases like cancer and other neurodegenerative disorders, wherein the removal of protein aggregates and damaged cellular components is critical.[261] It is indicated that nanomaterials cause disturbances in the signaling pathways, lysosome destruction, oxidative stress, etc., which modulates the autophagy driven suicide of the cells. However, there are also reports wherein nanomaterials enhanced the survival of the cells. Further research is needed in this direction to discover the exact underlying mechanism to harness the potential of nanomaterials in autophagy modulation.

5. CONCLUSION AND FUTURE DIRECTIONS

COVID-19 has caused havoc worldwide with trillions of dollars of losses and millions of deaths. There is an urgent need to find an effective solution to control the pandemic. This has boosted R worldwide and recent findings of high-resolution structure of 3CL protease and the 3D structure of the spike glycoprotein from SARS-CoV-2 have provided better understanding about the viral replication and pathogenesis, respectively. X-ray crystallographic studies of the spike protein reveal that it has some ridges which are more compact than the SARS-CoV, and responsible for adaptive and tighter binding to human ACE2 receptors. These advanced outcomes facilitate the development of vaccines and antivirals by correlating mutations. There are many reports wherein the therapeutic candidates show promising *in vitro* studies, while shows inefficacy or even cause fatal syndromes during *in vivo* studies and clinical trials. Consequently, more insights may be required for determining the effective binding pockets and finding efficient drug molecules. So, to be on the safer side, it is better to adopt broad-spectrum drugs and follow required precautions. It is also advised to look at the nanotechnological approaches for an improved theranostic deployable solution. In the past decades, there has been tremendous research on nanotechnology for increasing the efficacy of the therapeutics for various indications, which can be extended to COVID-19.

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Notes

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