Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Chapter 2

COVID-19 outbreak: comprehensive update on epidemiology, transmission, and treatment opportunities

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Chapter outline

2.1 Introduction

Tuesday, December 31, 2019 was the brink of the commencement of this global pandemic which brought the world to its knees. Twenty-seven incidents were reported in Wuhan located within the Hubei province of China [1]. These patients suffered from symptoms of pneumonia. However, in reality they grieved from the coronavirus disease (COVID-19) [2]. Earlier, an unidentified

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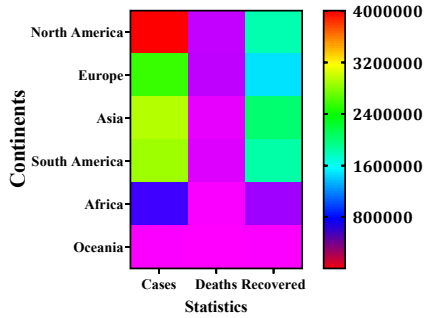

FIGURE 2.1 Statistics indicated by density map representing total cases, deaths, and total recovered with respect to continents till July 13, 2020.

type of pneumonia was discovered on December 12, 2019 and the possibility of viral infections was cleared by laboratory testing. On January 7, January 2020, the viral agent was obtained through the swabs taken from the market by the Centre for Disease Control (CDC) but till then the infection had spread rampantly and flooded the hospitals with a large number of cases [3]. The World Health Organization further designated the disease as "COVID-19" [4]. On January 22, 2020, the novel COVID-19 was found to be transmitted from bats and was categorized to the second group of β-coronavirus [5]. Fig. 2.1 indicates the statistical distribution in terms of a density map with respect to the total cases, deaths, and total recovered as per the continents. Color map indicates the density of population affected, deaths, and recovery from the infection with respect to different continents. It is observed that the numbers of cases are highest in Europe and North America while it is lowest for Oceania. It is also found that the number of deaths is less compared to the number of patients recovered. This indicates that the infection is controlled to a certain extent and the treatment is modest. In this chapter, we discuss the epidemiology, age demographics, comorbidities, genomic organization of SARS-CoV-2, transmission, replication, and potential therapeutic strategies therein.

2.2 Epidemiology, age demographics, and comorbidity status

Till July 13, 2020, a total of 13,042,340 cases have been reported for confirmed cases, out of which 571,689 patients have died and 7,588,510 patients have recovered from this viral infection [6]. Severely affected countries include India, the United States, Italy, China, Spain, Russia, Brazil, and the United Kingdom. The remaining countries have controlled the outbreak significantly and have slowed down its progression till now. This infection has about a 5.16% of mortality rate and a 53.73% chance of recovery [7]. Fig. 2.2A indicates the mortality rate corresponding to the age groups. In order

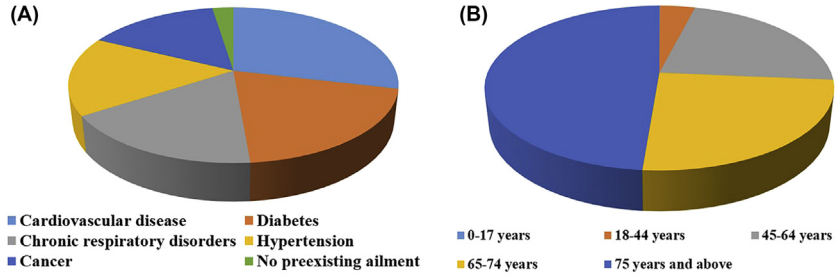

FIGURE 2.2 Age (A) and comorbidity (B) demographics correlated with mortality rate among the infected patients.

to trace out a pattern with the age demographics, it is essential to understand the effect of the infection on sex and comorbidities. The sex demographics indicate a 1.617% increased male death rate compared to the females. Highest death rate is seen in patients with cardiovascular diseases and diabetes followed by respiratory diseases, hypertension, and cancer [8]. Fig. 2.2B indicates the mortality rate by the infection with respect to various diseases.

2.3 Origin, cellular etiology, and pathogenesis

The name "corona" in Latin refers to "crown," which originated from the spikes on the viral coat resembling a crown-like appearance when observed under an electron microscope. Fig. 2.3 indicates structural features of SARS-CoV-2. The subfamily Orthocoronavirinae of the Coronaviridae family (order *Nidovirales*) classifies into four subfamilies—alpha coronavirus (α COV), beta coronavirus (β COV), delta coronavirus (Δ COV), and gamma coronavirus (γ COV) [9]. Till today, seven human COV (HCOV) have been discovered which are capable of infecting humans [10,11]. The HCOV can further be classified as follows:

a) **Common COV:** These include HCoV-OC43 and HCoV-HKU1 belonging to the β-COV subfamily; HCoV-229E and HCoV-NL63 of the α-COV

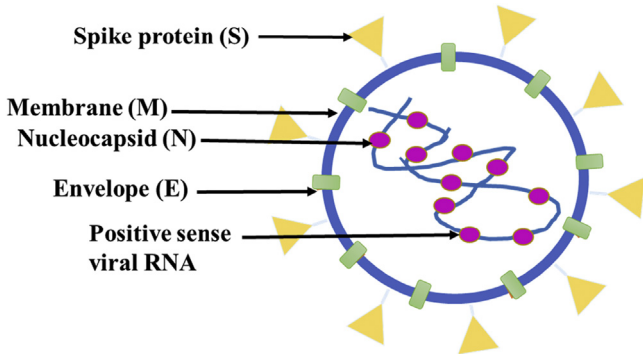

FIGURE 2.3 Structural features of SARS-CoV-2.

subfamily of lineage B, C, and D [12]. These may result into common cold infections and upper respiratory tract disorders in healthy patients; however, the infections are hardly contagious. The immunocompromised subjects and the elderly are susceptible to lower respiratory tract infections.

(b) **Rare COV:** These include SARS-CoV, SARS-CoV-2, and MERS-COV, which belong to the β-COV subfamily of lineage A. They have highly unpredictable and contagious clinical behavior introducing respiratory and other severe organ manifestations in patients across the world [13,14].

2.4 Genomic organization

The complete genetic composition of SARS-CoV-2 is composed of a single strain (positive sense) of RNA [15]. Its genome consists of a total of 29.9 kbp which code for 9860 amino acids [16,17]. The constitutional proteins encoded in the viral genomic RNA code for four proteins—spike (S), envelope (E), membrane (M), and nucleocapsid (N) [18]. About two-third of the viral RNA, localized in the ORF1a/b region, encodes for two subunits of polyproteins during the translation process (pp1a and pp1ab, respectively) and encodes for a set of 16 nonstructural proteins (NSP), whereas rest of them code for accessory as well as structural proteins [19]. ORF1a/b is responsible for encoding the papain-like protease and 3CL-protease among other enzymes. The ORF1b polyprotein codes for RNA-dependent RNA polymerase, helicase, and endoribonuclease enzymes, while the further region codes for spike, nucleocapsid, membrane, and envelope proteins [20,21]. The single mutation in the N501T segment of the spike protein may be predominantly involved in boosting its specificity for the human angiotensin-converting enzyme II (ACE2) receptor [22]. Wu et al. reported that the WHCV contained 16 predicted NSP by performing metatranscriptomic sequencing studies and phylogenetic similarity to SARS-CoV, especially in the S protein and the receptor-binding domain (RBD) [23–25]. This is the preliminary mechanism for its human transmission. Fig. 2.4 indicates the genomic organization of SARS-CoV-2.

2.5 Transmission

The evidence so far indicates rapid human–human transmission arbitrated via droplet route, i.e., coughing or sneezing from the infected patient with a droplet diameter ranging between 1 and 10 μm in diameter with a proximity between the individuals of about 1 m. Indirect contact with the infected surfaces or belongings of the patient post contact with the nose or mouth serves as a passage for the viral entry. Coronavirus have been associated predominantly with the respiratory tract of the infected patient [26]. Studies carried by other researchers found the presence of CoV in dead Malayan

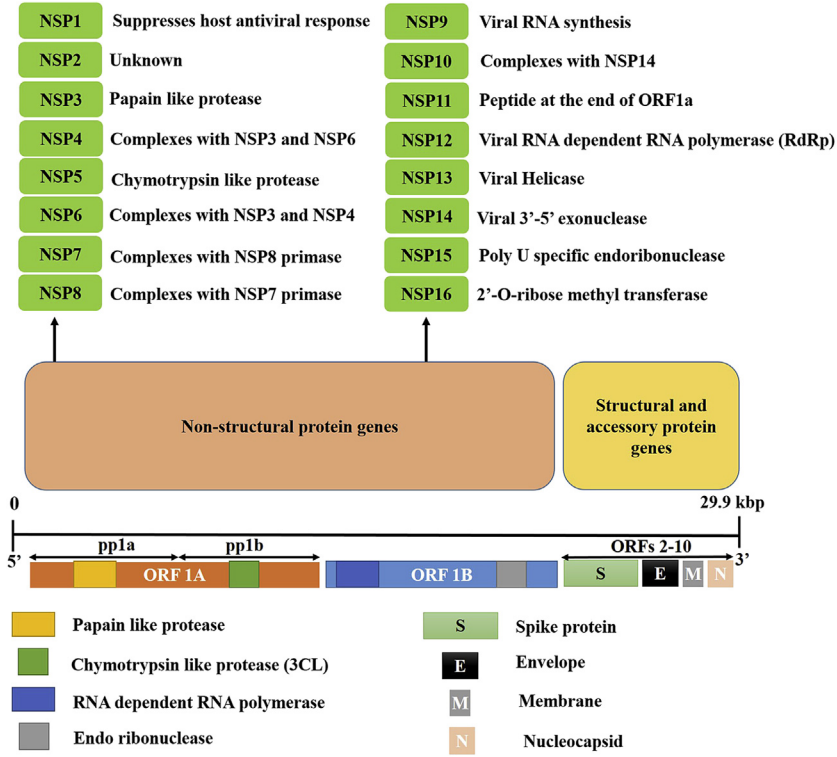

FIGURE 2.4 Genomic organization of SARS-CoV-2.

pangolins and designated it pangolin-CoV and reported a 91.02% similarity with the SARS-CoV-2. They also deciphered a close relationship between the RBD of S1 protein and its interaction with the ACE2 receptor, thereby making humans a host for SARS-CoV-2 infection [27]. Furthermore, studies carried out by Huang et al. indicated that the spike protein in bat CoV RaTGl3 showed a 97.43% similarity with SARS-CoV-2, implicating a potential bat–pangolin–human transmission [28]. The basic reproduction number (R_0) is a measure of an expected number of secondary infections transmitted by an infected individual in a susceptible population during the average infectious period [29]. It helps us to estimate the transmissibility of the infectious agents and determine the severity invasion characteristics and pathogenicity of the viral agent. It basically describes how contagious the infection in reality is [1,30]. Table 2.1 describes the basic reproduction with common viral infections which mankind has faced till now. The higher the reproduction number, the rapid is the transmission of the infection.

TABLE 2.1 Common viral infections and their basic reproduction numbers.

Viral diseases	Reproduction number (R0)	References
MERS	1.9	[31]
SARS-CoV-2	3.49	[32]
Seasonal flu	1.28	[33]
Swine flu	1.33	[34]
Ebola	1.51	[35]
Zika	4.3	[36]
Nipah	0.48	[37]
SARS	3.5	[38]
HIV	6	[39]
Mumps	6.54	[40]
Small pox	6.87	[41]
Measles	12	[42]
Polio	6	[43]
Spanish flu	2.2	[44]

2.6 Replication cycle

Replication cycle of SARS-COV-2 initiates when the S protein first comes in contact with the host ACE2. During this time, a change in the protein conformation occurs from closed to open conformation. This change in RBD confirmation initiates the clathrin-caveolae mediated endocytosis pathway which is pH-dependent. Acidic pH favors endosomal fusion and endocytosis of the virus across the cell membrane [45,46]. Post endocytosis event, viral genome is released into the host cell where it fuses with the ribosomes to conduct translation of viral proteins. The resulting complex initiates the RNA (-sense) synthesis with the help of replication and transcription processes [47]. At the time of replication, full length (-sense) RNA copies are created and utilized as a template for (+sense) RNA genomes synthesis. Furthermore, 7-9 subgenomic nested transcription takes place for specific proteins of the virus namely nucleocapsid (N), spike (S), membrane (M), and envelope (E), respectively. They are then transported to the golgi bodies where assembly of viral proteins leads to virion formation. The typical incubation time required by the SARS-COV-2 virus lies between 0 and 14 days [48].

2.7 Signs and symptoms

In order to investigate the clinical signs and symptoms, a study was performed by Guon et al. in China by analyzing 1099 confirmed cases. They reported that the clinical manifestations composed of fever in about 88.7%, cough in 67.8%, fatigue in 38.1%, sputum formation in 33.4%, wheezing in 18.6%, sore throat in 13.9%, and headache in 13.6% of the patients [49]. In adverse cases, the neutrophil count, blood urea, nitrogen, creatinine levels, hepatic enzymes, lactate dehydrogenase, and C-reactive proteins were found to be significantly higher than normal values. Additionally, inflammatory load by the cytokines such as various interleukins (IL-6, IL-10,IL-2, IL-7, IL-10), tumor necrosis factor α (TNF α), interferon and monocyte proteins, granulocyte colony-stimulating factor (GCSF), interferon γ—induced protein (IP-10), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein 1-α (MIP-1α) levels increase intermittently, depicting the active immune status of patients [50,51]. Guan et al. reported that the chest computed tomography imaging showed glass-like opacity and bilateral patchy shadowing with a spherical framework and a peripheral pulmonary distribution of the infection [49].

2.8 Potential therapeutic strategies

We have envisaged the entire pathogenic cycle from the entry, replication, and assembly to transmission along with the route of transmission. Each step of the cycle requires a certain set of enzymes which can be the potential targets to the researchers against the COVID-19 disease.

2.8.1 Entry

As discussed previously, the entry is the most primitive and important step of the viral infection. The viral S protein integrates with the ACE2 receptor on the human cell surface. A change in confirmation activates and mediates the endocytosis process. Some clinical success has been observed recently with chloroquine owing to the inhibition of this step. The underlying mechanism of chloroquine is to raise the pH of the endosome, thereby causing alkalization and preventing endocytosis [52]. A similar mechanism is reported to be followed by bafilomycin A, an antifungal drug, and omeprazole, a proton pump inhibitor [53]. This mechanism must be extrapolated to the antiviral activity of these drugs. Another prospective drug may be a selective ACE2 enzyme inhibitor such as the drug code PD 123177 which inhibits the ACE2, thereby blocking the contact of the S protein with the ACE2 receptor [54]. Ivermectin has also been recently approved by the FDA for treating COVID-19. It acts as an integrase inhibitor in HIV and importin IMP α/β1 heterodimer protein

preventing entry into the healthy cell [55]. Studies on SARS-CoV-2 have revealed an important role of IMP α/β1 in signaling dependent nucleoplasmic regulation of the nucleocapsid protein. Some other drugs having a similar activity in inhibiting ACE2 receptor and preventing the interaction between ACE2 and S protein are umifenovir (Arbidol) and camostat mesylate. Camostat mesylate inhibits a host type II transmembrane serine protease known as TMPRSS2, which enables the cellular entry via the S protein and targets the membrane fusion between the host cell and the viral cell [56,57]. Arbidol blocks the fusion of the viral membrane proteins with the host cell membrane, averting entry into healthy host cells [58]. It has been sanctioned in Russia and China for treating influenza with an intriguing interest for the treatment of COVID-19.

2.8.2 Translation and replication inhibitors

Without the RNA polymerase, the viral RNA may not be able to replicate inside the host cell. This also hinders the translation process by preventing the synthesis of viral proteins. Remdesivir, also recognized as GS-5734, a viral polymerase inhibitor, has gained a lot of importance in the present situation owing to its RNA polymerase inhibitory activity which prevents the viral RNA replication, translation, and viral protein synthesis as shown in Fig. 2.5, respectively. It is a monophosphate prodrug which metabolizes to an active adenosine nucleoside triphosphate analog and belongs to the class of adenosine nucleotide analogs of the viral polymerase inhibitors [59,60]. This agent was brought into the limelight during a screening study for antibiotic agents with inhibitory potential against RNA viruses such as Coronaviridae and Flaviviridae family. This agent showed promising activity against Ebola viral

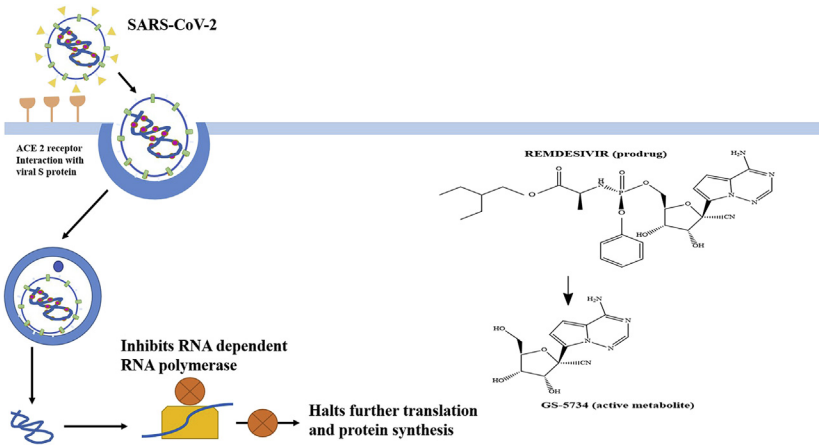

FIGURE 2.5 Mechanism of action for remdesivir.

outbreak attributed to its reduced EC_{50} and viral polymerase selectivity leaving the host unaffected [61]. Today, remdesivir is a breakthrough drug for COVID-19 owing to its broad spectrum and in vitro potency against several CoV and even SARS-CoV-2 with EC_{50} and EC_{90} values at 0.77 μM and 1.76 μM congruently [62,63].

Similar drugs include favipiravir, which exhibits antiviral activity by inhibiting RNA-dependent RNA polymerase enzyme. Favipiravir, also recognized as T-705, is also a prodrug of a purine nucleotide which is metabolized systemically to favipiravir ribofuranosyl-5′-triphosphate. This active moiety binds to RNA polymerase and stops viral replication. Favipiravir has showed a broad spectrum of antiviral activity in manifestations like influenza and Ebola which is extrapolated to the SARS family [64]. Lopinavir and darunavir have been proven to inhibit chymotrypsin like protease 3CL hindering the viral replication [65]. These drugs have been used earlier for the treatment of HIV by inhibiting HIV-1 protease enzyme [66]. The role of proteases exists at an advanced point in the replication cycle since the large viral polyprotein needs to be cleaved into various compartments for maturation of RNA. Chloroquine has proven to be a boon for the therapy of SARS-CoV-2 with underlying various mechanisms responsible for its proven antiviral activity. Although it was marketed earlier as an antimalarial drug, it has a cascade of mechanisms at almost every step of the viral cycle to hinder the viral processes. Initiating with the viral entry, clathrin, a surface protein, plays a critical role in the vesicle formation and viral cellular endocytosis [45,67]. Chloroquine has the potential to inhibit the formation of clathrin viral cell complex, thereby preventing endocytosis. Alkalinization of the acidic pH of the endosomal vesicles may help prevent the viral RNA discharge post endocytosis [52,68,69]. Chloroquine also has a role to play in inhibiting the MAPK signaling pathway, thereby halting viral transcription. Chloroquine also blocks the quinone reductase 2 enzyme, which plays an important role in the sialic acid synthesis pathway which is a transmembrane protein needed for ligand recognition. The failure in ligand recognition may prevent all the processes from viral entry to replication and translation, respectively [70,71]. Fig. 2.6 represents the mechanisms of action of chloroquine and hydroxychloroquine (HCQ).

HCQ is a hydroxyl radical of chloroquine. The hydroxyl group makes the moiety weakly basic and enhances the potency compared to chloroquine. The enhanced potency may be due to enhanced alkalinization of the endosomal vesicle compared to chloroquine [72]. Addition of the hydroxyl group renders reduced permeability to the blood−retinal barrier, promotes rapid clearance, and reduces QT interval prolongation, hypoglycemia, neuropsychiatric effects, and retinopathy, thereby implying reduced retinal toxicity compared to chloroquine [47,52,73,74]. Therefore, HCQ is a safer therapeutic option compared to chloroquine.

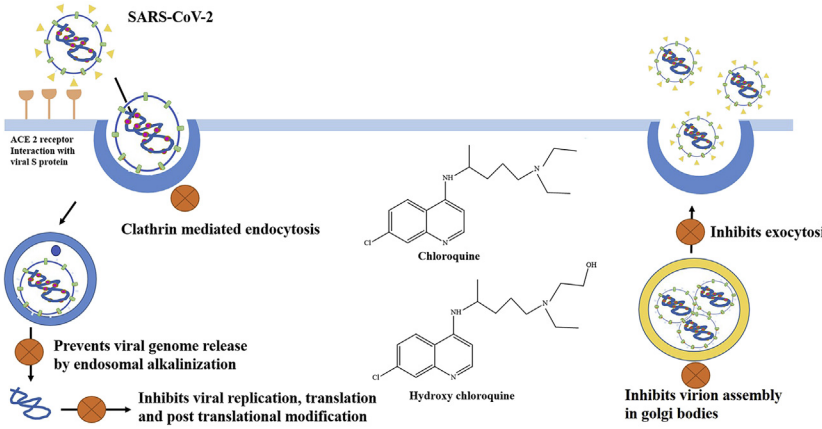

FIGURE 2.6 Mechanism of action of chloroquine and hydroxychloroquine.

2.8.3 Cytokine storm

The immune system of the infected patients reacts aggressively in response to the infection in the form of a cytokine storm through MAPK signaling pathway, T cell proliferation, elevated interleukins (IL-6, IL-10,IL-2, IL-7, IL-10), tumor necrosis factor α (TNF α), interferons, and monocytes [75]. A reduction in the CD28 cells and CD4 cells is observed in infected patients. Out of all such markers, interleukin-6 plays a crucial role in stimulating the cytokine storm in the lungs [76]. Monoclonal antibodies can be tuned with high macromolecular specificity and may prove to be constructive in treating the cytokine storm during the infection. Monoclonal antibodies targeting IL-6 may help to reduce the cytokine storm and ameliorate the clinical results. Tocilizumab is reported to have high affinity and selectivity toward IL-6, previously permitted by the FDA for treating rheumatoid arthritis and cytokine syndrome [77]. Clinically, 400 mg of tocilizumab was found to achieve therapeutic success in treating 21 patients with COVID-19, which showed clinical improvement in 91% of patients with an amended pulmonary functioning, rapid recovery, and successful discharge only with a single dose [78,79]. Sarilumab, a similar type of IL-6 receptor antagonist previously sanctioned for rheumatoid arthritis [80], is presently being utilized for treating adverse cases of COVID-19 in a multiple center double blind, in the phase 2 and phase 3 of the clinical trial. Some other monoclonal antibodies in clinical trials in China and the United States are vascular endothelial growth factor–specific bevacizumab [81] and fingolimod [82] and complement activation inhibited by eculizumab [83,84] respectively.

2.8.4 Immunoglobulin therapy

Administration of immunoglobulins may be associated with reducing mortality; however, it may not be sufficient to cure the infection. The process requires removal of large amounts of immunoglobulins or convalescent plasma from treated individuals, which is a time-consuming and expensive process [85,86]. The current marketed immunoglobulin products lack the protective antibodies to SARS-CoV-2. This process may not be amenable to scale up and only a limited number of patients can be treated at a time. The reproduction and transmission rate of the virus is several folds faster compared to the time consumed in the immunoglobulin therapy. The results may also vary depending on the immunity of the pretreated patients from which the convalescent plasma is taken [86]. Therefore, there is a need for a vaccine which can help in providing immunity toward SARS-CoV-2 and to eradicate this viral infection.

2.8.5 Vaccine development

Till date, no vaccine exists for combating SARS-CoV-2. At this stage, it is critical and imperative to develop clinically relevant vaccines to eradicate COVID-19. SARS-CoV-2 possesses substantial homology with SARS and MERS. Fig. 2.7 includes various approaches used for viral vaccine

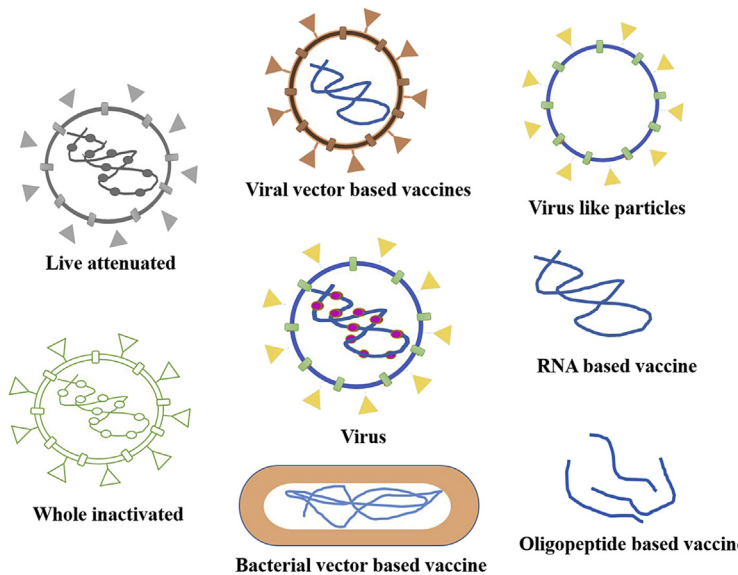

FIGURE 2.7 Approaches to viral vaccine development.

development. Patent application WO2015081155 includes the use of immunogens, isolated from the spike protein of the MERS-CoV virus for producing DNA-based vaccines. The spike protein stimulated humoral as well as cellular reactions which included an enhanced serum IgG titer levels. This reaction subsequently increased CD3+, CD4+, CD3+, and CD8+ T cell levels. Inovio Pharmaceuticals have publicized the designed DNA vaccine, designated INO-4800 on March 3, 2020. A patent application WO2010063685 by GlaxoSmithKline (GSK) unveiled a protein-based vaccine with the ability of promoting immune reaction against SARS virus family. It comprised of S protein used as an immunogen incorporated into an emulsion. This induced elevated amounts of SARS-CoV immunoglobulins 2a and 2b antibodies in animal models. GSK collaborated with Clover Biopharmaceuticals in February 2020. This alliance involved the testing of Clover's COVID-19 S-Trimer candidate within GSK's adjuvant. With the help of this Trimer-Tag technology, Clover synthesized an S-Trimer vaccine which was found to be superior compared to previous DNA-based vaccines. Another innovative approach used by Generex Patent application US20060002947 revealed the synthesis of amalgamated oligopeptides consisting of an invariant chain for antigen presentation, a structure connecting the key peptide to the antigen epitope along MHC class II binding epitope. Patent application WO2015042373 filed by Novavax revealed the constitution of MERS-CoV nanoparticles including S-Trimer protein, synthesized by a baculovirus while infecting Sf-9 cells. This virus-like particle approach promoted a strong antibody reaction in mice as well as transgenic cattle, post incorporation onto their patented adjuvant matrix M. Novavax declared that it initiated animal testing on prospective COVID-19 vaccines on February 26, 2020. The mRNA-based vaccines are advantageous in prophylaxis which possesses the capability to imitate a natural infection to generate a neutralizing immune reaction and renders the virtue of combining many mRNAs to formulate a solitary vaccine. Moderna therapeutics Inc. has reported that mRNA-1273 consists of a pre-fusion alleviated S protein linked to SARS-CoV-2. Vaccine repurposing has become a prime topic in fighting the battle against COVID-19. Among these vaccines, the Bacillus Calmette−Guérin which has been primitively used to prevent tuberculosis infection may play a significant role in regulating the morbidity and mortality rate associated with the pandemic owing to its broad spectrum immunogenic potential against several respiratory tract mediated infections, COVID-19 being one of them [87]. Table 2.2 indicates the vaccines currently under clinical trials.

TABLE 2.2 Vaccines presently under clinical trials.

NCT number	Interventions	Phases	Enrollment	Participating country
NCT04299724	Pathogen-specific aAPC	Phase 1	100	China
NCT04276896	Injection and infusion of LV-SMENP-DC vaccine and antigen-specific cytotoxic T lymphocytes	Phase 1/phase 2	100	China
NCT04313127	Recombinant vaccine using adenovirus type 5 vector	Phase 1	108	China
NCT04341389	Recombinant vaccine using adenovirus type 5 vector	Phase 2	500	China
NCT04334980	BacTRL-spike	Phase 1	84	Canada
NCT04324606	ChAdOx1 nCoV-19 MenACWY placebo	Phase 1/phase 2	510	England
NCT04283461	Biological: mRNA-1273	Phase 1	45	United States of America
NCT04336410	INO-4800 device: CELLECTRAÂ 2000	Phase 1	40	United States of America
NCT04368988	SARS-CoV-2 rS spike protein nanoparticle vaccine with/without adjuvant	Phase 1/phase 2	131	Australia
NCT04380532	Tableted V-SARS vaccine	Phase 1	20	Canada
NCT04368728	BNT162a1 (viral infection) BNT162b1 (respiratory viral diseases) BNT162b2 (viral infection) BNT162c2 (adverse reaction)	Phase 1/phase 2	7600	United States Germany
NCT04276896	Lentiviral vector system (NHP/TYF) to modify and activate dendritic cells and T cells	Phase 1/phase 2	100	China
NCT04357028	Measles, mumps, and rubella vaccine	Phase 3	200	Egypt

2.9 Conclusion and future perspectives

In this critical time of pandemic crisis, we endeavor to reveal some potential postulates regarding the morphology and epidemiology of contagious viral infection spread and progress with age and disease conditions. We have scrutinized the genomic compositional organization and replication cycles in detail along with the possible explored and unexplored targets throughout the viral processes. We have also explored and explained the significance of reproduction number "R_0" in transmission and its signs and symptoms of COVID-19 for symptomatic treatment. Current book chapter has traversed several potential therapeutic strategies both explored and waiting to be explored till date and various approaches for viral vaccine development. Through this prospective and comprehensive review, we would like to reach out to the readers emphasizing the capabilities and potential of current novel research trends to enlighten the ray of hope for the mankind to survive this critical crisis of COVID-19 pandemic.

List of abbreviations

ACE2 Angiotensin-converting enzyme II
CD Cluster of differentiation or cluster of designation or classification determinant
COVID-19 Coronavirus disease 2019
EC_{50} Effective concentration (half maximal)
FDA Food and drug administration
HCQ Hydroxychloroquine
IL Interleukin
MAPK Mitogen-activated protein kinase
MERS Middle East respiratory syndrome
MERS-CoV Middle East respiratory syndrome coronavirus
mRNA Messenger RNA
NSP Nonstructural proteins
R_0 Reproduction number
RBD Receptor-binding domain
SARS-CoV-2 Severe acute respiratory syndrome coronavirus-2
TNF α Tumor necrosis factor α
WHCV WH-Human 1 coronavirus

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