Chapter

Different Therapeutic Strategies to Tackle the Infection Associated with COVID-19

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Abstract

Covid-19 is a pandemic and the whole world is facing the loss in terms of morbidity and mortality of the human resources. Therefore, there is an urgent need for various therapeutic agents or drugs to treat the covid-19 patients. Although, vaccination process is under way, it is not possible to provide the vaccination to whole world in a short period. Therefore, it is an essential strategy to work on the various therapeutic aspects of covid-19 treatment. The present book chapter will discuss and review the various aspects of the treatment strategies of the covid-19. Further, we will provide an overview of the virus and host based potential therapeutic targets along with existing therapeutics which are effective against SARS-CoV-2 virus. Also, the novel vaccines are being developed against covid-19 deadly virus will be discussed.

Keywords: SARS-CoV-2, covid-19, therapeutics, pandemic

1. Introduction

The new covid-19 pandemic was reported in Wuhan, China in December, 2019 [1]. This disease is caused by corona virus also known as SARS-CoV-2 and characterized by severe acute respiratory distress syndrome [2]. As per the recent report of WHO on 20 December, 2020, there have been over 75 million cases and 1.6 million deaths reported worldwide since the start of the pandemic [3]. In 2002, SARS-CoV (Severe Acute Respiratory Syndrome Coronavirus) outbreak was reported in China then spread worldwide, whereas, MERS-CoV (Middle East Respiratory Syndrome Coronavirus) emerged in Saudi Arabia in 2012 with 37% mortality rate. Similar to SARS and MERS, newly identified severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) belongs to beta-coronaviridae family and showed close resemblance with them [4]. These three zoonotic viruses have pandemic potential and able to produce severe respiratory infection in humans [5].

The SARS-CoV-2 infection is transmitted through respiratory secretions either in droplet or aerosol form from one person to another [6]. Apart from respiratory secretions, urine, stool and close proximity with patients may be the sources for dissemination of SARS-CoV-2 [7, 8]. Based on phylogenetic studies, the genomic sequence of SARS-CoV-2 virus is 96% similar to bat corona viruses so these bats may be potential reservoir host for human corona virus [9]. However, there are

no clear evidences which have suggested virus transmission directly from bats to human population [10]. Further, some studies suggested that pangolins can be considered as intermediate hosts between bats and human [11, 12]. Severe infectious covid-19 cases have rapidly progressed to dyspnoea, shock and acute respiratory distress [13]. In addition, the other organ dysfunctions have also been reported from patients including severe cardiac injury, acute renal, gastrointestinal, liver injury, neurological defect along with coagulation impairment and death [13].

It is important to understand the virus biology, and replication cycle to identify effective therapies against SARS-CoV-2, because most of therapies are directly targeting the stages in the virus life cycle. Highly pathogenic SARS-CoV-2 are enveloped, single-stranded positive sense RNA betacoronavirus with size ranging from 80–120 nm, and their genomes encode non-structural proteins (nsps), structural proteins, and several accessory proteins [14]. Genome of RNA virus contains ten open reading frames (ORF1–10) and has total 29,903 nucleotides [15]. Among the ten ORFs ORF2–10 generates four structural proteins S (spike), N (nucleocapsid), E (Envelop protein), M (Membrane protein) along with auxillary proteins. However, large replicase polyproteins (PP1a/b) encoded by ORF1ab further gets cleaved by proteolytic enzymes into non structural proteins (nsp1–16) [15].

The SARS-CoV-2 virus entry in host cell is mediated with attachment of the spike (S) glycoprotein with the host angiotensin-converting enzyme 2 (ACE2) receptor thereby infection process starts [16]. Further, virus S protein cleaved by the cathepsin L proteases which get activated in a pH- dependant manner allows the release of viral genome into host cell cytoplasm [17]. In addition, other host cell proteases like TMPRSS2 (Transmembrane Protease Serine Type-2) and TMPRSS11D (Airway trypsin like protease) participate in the cleavage of spike protein into its constituents (S1 and S2) which further lead to entry of virus genome into host cell through non endocytic pathway [18]. S1 subunit of spike protein possesses receptor binding domain (RBD) which binds with host receptors and S2 subunit favors fusion of viral membrane with host cell [19]. Once the virus genome released inside the host cell, then host ribosomes are involved in the process of translation of virus genome containing ORF1ab into replicase polyproteins PP1ab [20]. These PP1ab further cleaved by important viral proteases include 3CLpro (3 chymotrypsin like proteases) and PLpro (papain like proteases) to generate nsp2–16 [20]. These nsp2–16 are participated in virus replication and transcription complex, while virus structural proteins are translated from another ORF2–10 containing viral genome and contribute to outer structure of virus [21]. At last, the newly born virions are delivered outside the infected cell by exocytosis after completion of their structural assembling in the endoplasmic reticulum golgi bodies complex [22].

The WHO (world health organization) has declared covid-19 a public health emergency due to its high spreading potential across the world. Although, vaccine development trial has almost finished and vaccination drive is going to be started. However, till now there are no effective therapies or specific drug candidates against this communicable disease. Thus, it is required to understand detail biology of virus (SARS-CoV-2) to further elucidate novel drug therapeutics.

2. Therapeutic agents to tackle the covid-19 infection

2.1 ACE-2 modulators

Like SARS-CoV, it is confirmed that SARS-CoV-2 virus also interacts with ACE-2 human enzyme for entry and replication into the host cell. SARS-CoV-2 spike protein has high binding affinity with ACE-2 enzyme present in respiratory epithelial

cell of host [23]. Hence, the therapeutics which inhibit spike protein-ACE-2 interaction would be considered effective therapy against SARS-CoV-2. ACE-2 enzyme is a zinc metalloproteinase which contains two domains in its structure which include amino terminal domain and carboxy terminal domain [5]. ACE-2 enzymes are exhibits in type-I and type-II alveolar cells of respiratory tract, liver, kidney, testes, heart and intestine [24]. Wu et al. [25] have found that ACE-2 enzymes are highly expressed in alveolar epithelial type-II cells in an around 83% which indicate these cell can be served as reservoir for virus. ACE-2 enzyme is a key regulator protein of RAS (Renin-Angiotensin System) system which contributes to vascular homeostasis [26]. In RAS system, angiotensinogen glycoprotein is cleaved by renin enzyme present in kidney to angiotensin-I, which has converted into angiotensin-II (Ang-II) by ACE-1. Further, Ang-II binds to angiotensin receptor (ATR1) and produces vasoconstriction, cell proliferation, inflammation, thrombosis and vascular constriction [27]. For the counterbalance of AngII- ATR_1 axis effect, AngII is cleaved by ACE-2 enzymes into Ang1–7 peptides [28]. These angiotensin peptides further act on MASR (mitochondrial assembly receptor) and exhibits protective effects such as anti-inflammatory, anti-apoptotic and vasodilatation. Rothlin and co-worker [29] have reported the protective effects of ACE inhibitors and angiotensin receptor blockers (ARBs). They have found that patients with Covid-19 infection are taking ACE inhibitors and angiotensin receptor blockers exhibited lower mortality as compared with non-user patients. Previous study revealed that SARS-CoV virus down-regulates the ACE-2 enzymes present in host cell surface and increased ACE enzyme activity which lead to severe lung injury [30]. Increased ACE enzyme activity has been observed in SARS-CoV-2 patients. Hence, it has been proposed that delivery of soluble ACE-2 recombinant protein compete with host ACE-2 enzymes for the SARS-CoV-2 spike protein and ultimately neutralize the virus load and further, protect the patients from lung injury. For this purpose, recombinant human ACE-2 such as APN01 and GK2586881 have been analyzed and found effective in patient suffered from acute severe respiratory syndrome (**Figure 1**) [31].

2.2 TMPRSS2 inhibitors

Transmembrane serine protease-2 (TMPRSS-2) is present in host epithelium cells of various tissues [32]. It is involved in the pathogenesis of SARS-CoV-2 through cleavage of spike protein and facilitates virus entry into host cell [33]. Matsuyama et al. [34] demonstrated that over-expressed protein TMPRSS-2 containing vero E6 cell lines are susceptible for SARS-CoV virus infection and used as pharmacological tool for SARS-CoV-2 research. Thus any drug candidate which inhibits TMPRSS-2 protease may be effective in SARS-CoV-2 infection. In this regard, *in vitro* study conducted against SARS-CoV-2 to check the efficacy of compound camostat mesylate blocked the spike mediated virus entry in caco-2 cells [35]. In addition, some repurposing studies have been conducted to evaluate the efficacy of other proteases inhibitors such as nafamostat, 4-(2 aminomethyl) benzenesulfonyl fluoride and mucolytic drug bromhexine which can offer new therapeutic option for this pandemic (**Figure 1**) [36, 37].

2.3 JAK–STAT inhibitors

In covid-19 infection, patients suffer from severe acute respiratory syndrome in which huge amounts of various cytokines are released from immune cells leading to multiorgan failure and death [38]. Moreover, JAK–STAT signaling pathway is involved in SARS-CoV-2 virus entry into host cell which has linked with AAK1 (Adaptor-associated protein kinase-1) related clathrin mediated endocytosis [39].

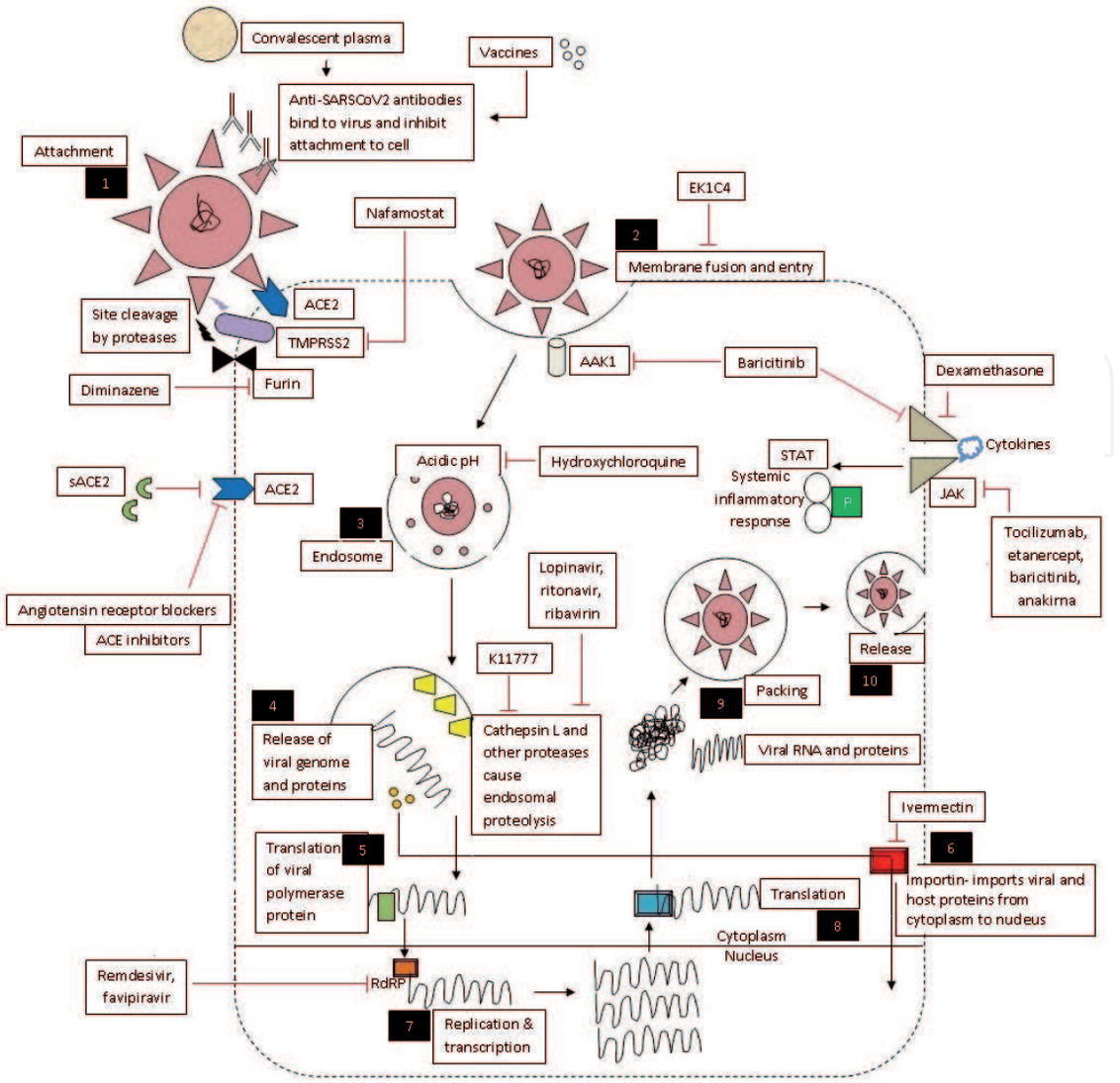

Figure 1.
Diagram showing the therapeutics approach to treat the infection of covid-19 patients.

Activation of JAK–STAT pathway through Ang-II/AT_1R may generate cytokines storm including IL-1, IL-2, IL-6, IL-7, IL-10 and TNF-α [39]. Thus, it may be suggested that JAK–STAT inhibitors could be potential therapeutics for the covid-19 infection. Further, Junior and co-workers [40] have shown that baricitinib, a JAK–STAT inhibitor, has potential to prevent generation of cytokines through inhibition of JAK–STAT signaling and also block the entry of SARS-CoV-2 via inhibiting AAK1 related clathrin mediated endocytosis (**Figure 1**).

2.4 Cathepsin L inhibitors

Cathepsin L is a cysteine protease reside in endosomes and works in acidic pH [41]. It cleaves S1 virus spike glycoprotein and facilitates three actions including virus entry into host cell, virus-host cell endosomal membrane fusion and RNA release. Serine protease (TMPRSS-2) acts on the surface of host cell membrane in neutral pH [42], whereas, cathepsin L mediates its action at host cell membrane as well as inside the endosomes in acidic pH [43]. Therefore, Liu and coworkers, [17] have proposed that the combined use of serine protease and cathepsin-L inhibitors could be effective therapeutics to prevent virus entry and their genome release inside the host cell thereby inhibit the virus replication. In addition, they have also mentioned some cathepsin-L

inhibitors compounds which are found effective against coronavirus infection such as dec-RVKR-CMK, K11777, small molecule 5705213, MDL28170, SSAA09E1, EST, and oxocarbazate. Based on the proposed mechanism, it is reported that the combined use of TMPRSS-2 inhibitors including camostat and nafamostat mesylate along with cathepsin-L inhibitor E64d have shown inhibitory potential against SARS-CoV and SARS-CoV-2 infection in human epithelial cells (**Figure 1**) [44].

2.5 Furin inhibitors

Furin, is a human protease enzyme, present in multiple tissues and highly expressed in lungs [45]. It has been reported that SARS-CoV-2 virus contains furin like cleavage site (FCS) in its spike protein which has made covid-19 virus more pathogenic in nature as compared to other ancestors virus of coronaviridae family [46]. SARS-CoV-2 virus utilizes host furin protease for the cleavage of spike protein and gain entry into the host cell. Moreover, mortalities from SARS-CoV-2 infection have been reported in those patients compromised with cardiac disease, diabetes, obesity and hypertension and likely to be associated with higher circulating furin level [47]. Hence, scientists have drawn attention towards furin inhibitors to provide new therapeutic intervention against covid-19 infection (**Figure 1**).

2.6 Inhibitors of virus structural proteins and enzymes

Structural proteins and virus encoded enzymes of SARS-CoV-2 may be considered as important drug targets because these are responsible for virus survival and propagation. Researchers and pharmaceuticals companies have focused to develop short interfering RNA (siRNA) based therapeutics to target virus structural proteins and enzymes such as 3CL pro, PLpro and RNA dependant RNA polymerases to combat covid-19 infection [48]. Some previous studies revealed that siRNA therapeutics have already been designed against SARS-CoV and MERS viruses and found effective in outbreaks [49]. In addition, heptad repeat 1 region (HR1) present in the S protein involved in fusion and entry of virus could also be a good target for the development of fusion inhibitors against covid-19 [50]. Xia and co-workers, [51] have developed EK1C4 fusion inhibitors which target SARS-CoV-2 spike protein and found effective in HCoV-OC43 challenged mice. Recently in clinical trials, many chemical peptides, existing drugs and new drug candidates have been recognized through virtual and high throughput screening techniques against SARS-CoV-2 coded enzyme proteases [52]. Based on computational strategy, 6LU7PDB compound has been identified which acts as non-covalent inhibitor of 3CL pro enzyme in SARS-CoV-2 infection [53]. Some 3CL pro enzyme inhibitors antiviral drugs such as lopinavir and ritonavir have been found effective against SARS-CoV-2 virus infection [54]. In addition, some therapeutics are also identified which showed high binding affinity with SARS-CoV PLpro enzyme such as ribavirin, valganciclovir, beta-thymidine and some natural products like platycodin D, baicalin and catechin [55]. Moreover, RNA dependant RNA polymerase (RdRp) inhibitor antiviral drug remdesivir was approved by FDA for emergency treatment for covid-19 patients [56]. However, still its role is controversial for the treatment of covid-19 patients (**Figure 1**).

2.7 Inhibitors of cytokines

During covid-19 infection, higher amounts of cytokines have secreted from inflammatory cells and serve as potential therapeutic targets for drug development.

The major cytokines, IL-6, IL-1, TNF and interferons are generated during cytokine storm which cause the increased vascular permeability, vascular leakage along with dissemination of virus which may lead to fatal pneumonia and acute respiratory distress syndrome [57, 58]. However, many neutralizing strategy against these inflammatory mediators are being used to cope with this cytokine storm in covid-19 pandemic. Chi and co-workers, [59] reported the use of antibodies against IL-6 receptor (tocilizumab and sarilumab) for the treatment of covid-19 infection. Anti-TNF drug etanercept has shown favorable effect in covid-19 patients [60]. Moreover, another targeting approach against cytokine IL-1 is important because it is the major cytokine present in higher amount in alveolar lavage of covid-19 patients and secreted from inflammatory macrophages and monocytes [61]. Cavalli & coworkers, [62] have reported the use of anakirna in high dose and found safe in 72% patients suffered from covid-19 and ARDS with non-invasive ventilation outside the ICU. Furthermore, interferons (IFNs) have immunostimulant and antiviral effects and their use as a treatment along with some antiviral drugs have been found effective against MERS, SARS and IBV viruses (**Figure 1**) [63].

2.8 Antiviral drugs

As per the previous information related with SARS & MERS outbreak, many existing anti-viral drugs are being repurposed against SARS-CoV-2 virus in covid-19 pandemic. Remdesivir is a prodrug that converts into active metabolite and inhibits RNA dependant RNA polymerases (RdRp) thereby preventing the viral RNA synthesis [64]. It is prescribed against ebola virus infection and reported to have *in vitro* antiviral activity towards SARS and MERS coronaviruses [56]. Recently, it was reported that remdesivir prevents SARS-CoV-2 infection in human liver cancer cells [65]. Based on one clinical trial, remdesivir has been found clinically effective in 36 out of 53 patients suffered from covid-19 infection and receiving oxygen support [66]. In addition, many clinical trials are being carried out to check efficacy of remdesivir in covid-19 patients in various countries. Hung & co-workers, [67] have conducted clinical trial by using the combination of triple antiviral drug include lopinavir, ritonavir and ribavirin along with interferon which were found more promising compared to antiviral drug used alone in patient suffered from covid-19 infection. Another antiviral drug, favipiravir inhibits viral RNA polymerase enzyme and reported to have antiviral activity against many RNA viruses such as influenza, bunya and filoviruses [68]. However, to check the clinical efficacy of favipiravir in covid-19 patients various clinical trials have been performed in China and found favorable results (**Figure 1**) [13].

2.9 Corticosteroids

Corticosteroids are extensively used for SARS-CoV, MERS-CoV, H1N1 influenza, and ARDS that have similar pathological features with covid-19. But, their role in reducing mortality and improving these conditions remain controversial [69]. It was reported that corticosteroids did not improve the outcome during the SARS and MERS outbreaks, but delayed viral clearance and increased rates of secondary infections [70]. A systemic review and meta-analysis are conducted in covid-19 patients by van-Paassen et al. [71], their findings based on observational and clinical studies suggested the beneficial effects of corticosteroids on mortality rate and reduced ventilation support. However, delayed viral clearance and increased secondary infection have also been observed. Similarly, the study has been conducted in China in 201 patients confirmed with covid-19 pneumonia. In 62 patients who received methylprednisolone likely had decrease risk of death [72]. As per

another report of Mishra & Mulani, [73] corticosteroids are not recommended in the late course of acute respiratory distress syndrome (ARDS) condition because their persistent use more than 2 weeks has increased risk of death in ARDS patients. It seems that corticosteroid treatment work like double edged sword in covid-19 fight, therefore duration of corticosteroid therapy needs to be clarified in clinical trials (**Figure 1**).

2.10 Convalescent plasma therapy

Convalescent plasma is obtained from donors who have recovered from covid-19 infection, possess antibodies against SARS-CoV-2 that may neutralize the virus and modify the immune response [74]. Convalescent plasma therapy offers short term protection strategy and generates immediate immune response in susceptible patients. This approach has already been used in earlier outbreak of corona viruses such as SARS and MERS [75]. In this corona pandemic, many clinical trials have also been conducted against SARS-CoV-2. Recently the clinical study conducted by Duan et al. [76] revealed that the convalescent plasma therapy was well tolerated and positively improved the clinical outcomes in severe covid-19 patients. Salazar et al. [77] reported the use of convalescent plasma therapy in 25 patients who had severe covid-19 disease and evaluated safety along with clinical outcomes at 14 day after the transfusion. They found clinical improvement in nine patients within seven days and other were discharged at day 14. Hence, convalescent plasma therapy has potential to treat covid-19 cases but some adverse events have also been reported such as allergic reaction, dyspnoea and acute lung injury [78] (**Figure 1**).

2.11 Ivermectin

Ivermectin is an broad spectrum antiparasitic drug and its antiviral activity has also been reported against number of viruses both *in vitro* and *in vivo* [79]. Recently, *in vitro* study revealed that ivermectin can inhibit SARS-CoV-2 replication by reducing viral RNA up to 5000 fold at 48 h. in culture cells [80]. However, the mechanism of action of ivermectin is not clearly known. Choudhry and Sharma, [81] have mentioned that ivermectin may act by creating acidic environment and blocking the importin IMP2/β1 mediated viral intranuclear import (**Figure 1**).

2.12 Hydroxychloroquine

Hydroxychloroquine has been prescribed since decades in the prevention and treatment of malaria as well as rheumatoid arthritis and systemic lupus erythematosus (SLE) like chronic inflammatory condition [82]. In SARS-CoV-2 pandemic, it was suggested that hydroxychloroquine may have potential to treat covid-19 affected patients [83]. Food and Drug Administration (FDA) has granted permission for emergency use of hydroxychloquine in the treatment of covid-19 patients during initial stage of pandemic [84]. Recently, many *in vitro* studies have been conducted and found that hydroxychloroquine possess inhibitory activity against SARS-CoV-2 [85, 86]. Further, several studies have been published regarding the use of hydroxychloroquine in covid-19 but results are conflicting. One population based cohort study conducted by Rentsch and coworkers, [87] stated that there was no difference observed in mortality of covid-19 patients who had already received hydroxychloroquine for the treatment of rheumatoid arthritis or systemic lupus erythematosus. Similarly, in an observational study in covid-19 hospitalized patients, hydroxychloroquine did not show any benefit over mortality reduction (**Figure 1**) [88].

2.13 Vaccines

Various vaccine developments are being carried out across the world due to urgent need to overcome this covid-19 pandemic. Now in covid-19 emergency, vaccines could only be considered as potent therapeutics against SARS-CoV-2 deadly virus which normalize social life and working environment as it was earlier before pandemic. For the effective vaccines development against SARS-CoV-2 virus, various components are being used which include inactive or live-attenuated viruses, virus-like particles, viral vectors, protein-based, DNA-based, and mRNA-based vaccines [89]. Till now various potential vaccine candidates have already been

Type/ plateform	Vaccine Construct	Developer	Clinical Stage & Current Status	References
Inactivated virus	Nucleic acid	National institute for communicable disease control and prevention, China	Phase-III	[91]
Inactivated virus	Novel corona virus inactivated vero cells	Bejing institute of biological products Sinopharma	Ongoing Phase-III	[92]
Inactivated virus	Whole virion inactivated SARS-CoV-2 antigen	Bharat Biotech, India	Phase-I / II (DCGI-CDSCO Approved for emergency use in India)	[93, 94]
DNA	S Protein	INOVIO Pharmaceuticals, with, International Vaccine Institute and Seoul National University Hospital of South Korea	Phase-I/ II (Phase–III study put on hold by FDA,US)	[95, 96]
RNA	LNP-encapsulated m-RNA	Moderna with national institute of allergy and infectious disease, USA	Phase-III [FDA issued Emergency Use Authorization (EUA)]	[97, 98]
RNA	Lipid nanoparticle encapsulated mRNA (BNT162b2)	BioNTech and Pfizer	Phase-III (FDA issued EUA)	[99]
Protein subunit	Full length SARS-CoV-2 glycoprotein nanoparticle vaccine	Novavax, USA	Phase-III (Planning to apply FDA EUA in April, 2021)	[100, 101]
Non replicating virus vector	ChAdOx1-S	University of Oxford with Astrazeneca (UK) and Serum Institute, India	Phase-III (Approved for emergency use in some selected countries)	[102, 103]
Non replicating virus vector	Adeno virus based	Gamaleya Research Institute (Sputnik U)	Phase-III (Approved for use in Russia)	[104]

Table 1.
List of various important vaccines which are developed or being in different development phases against covid-19 infection.

developed and undergone for vaccination shot which have completed their clinical evaluation phase successfully [90]. However, several new vaccines are still under clinical developmental phase (**Figure 1**). Some important vaccines which are being developed against SARS-CoV-2 virus are mention below in **Table 1**.

3. Conclusion

Covid-19 is a devastating situation to the whole world and this infection is the reason for the morbidity and mortality of millions of people around the globe. It has shown impact on the health, economy and social aspect of the general population. Various therapeutic agents like ACE-2, TMPRSS2, JAK–STAT, cathepsin L, furin inhibitors, antiviral drugs, corticosteroids and plasma therapy have been tried for the treatment of covid-19 infected patients; however, conflicting results are obtained during the various clinical trials in the use of some therapeutic agents. Further, various vaccination programmes through various vaccine candidates are under progress; nevertheless, it will take time to complete dosing the millions of people. Therefore, various therapeutic agents are in need and require research to tackle this SARS-CoV-2 infection.

Conflict of interest

The authors declare no conflict of interest.

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